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A SIMPLIFIED SENSITIVITY PROCEDURE FOR ANAEROBIC MICRO-ORGANISMS*

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INTRODUCTION

Several months ago a patient, Mrs. R., came to us with an aggravated cyst in the lower spine region for aerobic and anaerobic culture and sensitivity. Clinically the patient was in much discomfort and the affected area was quite painful. Aspiration consisting of a serosanguinous material and pus was obtained by means of a syringe and needle for culture. The specimen was immediately plated to two fresh blood agar plates for aerobic and anaerobic studies; one tube of brain heart infusion broth and one thioglycolate broth (without indicator) were inoculated. The direct stain revealed many pus cells and a few small Gram positive cocci which did not appear to chain.

The culture showed no growth in twenty-four hours with the exception of the thioglycolate tube, which was turbid, but showed a clear zone to a depth of about three-fourths of an inch. This tube was subcultured to brain heart infusion broth, thioglycolate broth, and to three blood agar plates. The plates were incubated aerobically, under approximately 10% CO_2 tension and anaerobically. Two days after the patient had come in to us, it was apparent that the organism did not subculture readily to solid media since our plates showed no growth after twenty-four and forty-eight hours incubation and that the microbe was not an aerobe, for our brain heart infusion broth remained clear. Since the thioglycolate tube appeared to be a pure culture as evidenced by a Gram stain, a plate sensitivity was attempted. We soon found that the micro-organism would not grow readily on solid media regardless of the size of inoculum, although growing luxuriantly in thioglycolate broth in twenty-four hours. Our streaked plates showed extremely sparse growth in six to seven

* Second ASMT Award. Read before ASMT, June, 1955, New Orleans, La. Also second Scientific-Products Foundation Award, Bacteriology Division, 1955.

days under anaerobic conditions. No growth appeared aerobically or under reduced O_2 tension and our plate sensitivity could not be interpreted. This was the problem confronting us.

It is a well known fact that *in vitro* bacterial sensitivity to drugs, be they antibiotic or chemical as furadantin, is a relative tool and is not exactly transferable to *in vivo* conditions. Our plate disc sensitivity testing is a rough draft by which the attending physician can begin therapy. Why not then, equip the physician in this case with such a rough draft. The plate disc sensitivity was tried without success, so a quick simple tube sensitivity was needed.

We identified the organism as an anaerobic gamma *Streptococcus* which grew sparingly or not at all on solid media, but quite well in thioglycolate broth. This identification was confirmed by Communicable Disease Center, Chamblee, Georgia. The time-consuming standard tube dilution sensitivity procedure is not a routine determination carried out in this laboratory. Also, many of the antibiotics needed were not on hand for this assay. This seems to be the case in many other laboratories since the advent of commercial discs.

PROCEDURE

1. Tube Disc Method:

The Thioglycolate medium was dispensed in 4 ml amounts in 13 x 100 mm tubes and autoclaved. A series of tubes was set up and a disc or tablet of the drug was dropped into the medium asceptically. The tubes were then refrigerated for approximately 2 hours to allow dissolution of the drug and then inoculated with the test organism. This first series of the tube disc method was inoculated with two drops of twenty-four hour broth culture of the test organism, incubated at 37° C for twenty-four hours and the sensitivities read; we reported as sensitive only those tubes which showed no growth.

Since the first set up, we modified this procedure to comply as closely as possible to the tube dilution test as follows:

Thioglycolate medium is hydrated, dispensed in 100 ml. amounts and autoclaved. Two and one-half ml. of broth are transferred asceptically into 15 x 85 mm tubes, incubated twenty-four hours at 37° C for sterility and then they are ready for use. Difco's highest concentration discs are dropped into the fluid, the tubes are refrigerated for two hours and then 2.5 ml. of the same dilution as used in the tube dilution procedure is used for inoculum. The tubes are shaken, incubated at 37° C for twenty-four hours and read for complete inhibition.

2. Tube Dilution Method*

A correlation of this simplified procedure was made with the

* Chas. Pfizer and Co.

lengthy tube dilution assay. Using asceptic technique, stock solutions of the drugs* were diluted with brain heart infusion broth (Difco) to contain one thousand units or micrograms per ml. except Bacitracin. This antibiotic was diluted to contain 100 units/ml. and served as both stock and working solution. These stock solutions were dispensed into sterile tubes in one ml. amounts and immediately used or deep frozen. The stock solutions were thawed only once. We considered all frozen stock solutions outdated after one week.

One part of stock solution was diluted with four parts of broth to obtain the working solution. Except for the initial solution of stock, thioglycolate medium without indicator (BBL) was employed in all use of broth. Serial dilutions were performed as indicated in Chart I. The inoculum consisted of a 1-10,000 dilution in broth of an 18-24 hour culture of the test organism grown at 37° C. In the case of fastidious organisms, a much lower dilution of inoculum must be used (1:200 or 1:100). This was the case with the anaerobic *Streptococcus* in this series.

Chart I
Tube Set-Up

Tube No.	Diluent (Broth) Added	Drug Added	Diluted Culture Added	Final Drug Conc. in Units or Micrograms
1	None	2.5 ml. Working Solution	2.5 ml.	100
2	2.5 ml.	2.5 ml. Working Solution	2.5 ml.	50
3	2.5 ml.	2.5 ml. from Tube No. 2	2.5 ml.	25
4	2.5 ml.	2.5 ml. from Tube No. 3	2.5 ml.	12.5
5	2.5 ml.	2.5 ml. from Tube No. 4	2.5 ml.	6.25
6	2.5 ml.	2.5 ml. from Tube No. 5	2.5 ml.	3.125
7	2.5 ml.	2.5 ml. from Tube No. 6	2.5 ml.	1.56
8	2.5 ml.	2.5 ml. from Tube No. 7	2.5 ml.	0.78
9	2.5 ml.	2.5 ml. from Tube No. 8†	2.5 ml.	0.39
10	2.5 ml.	None	2.5 ml.	Zero

† Discard 2.5 ml. from Tube No. 9.

The test series were incubated at 37 degrees C for twenty hours and read for growth. Growth or a plus-minus growth was considered resistant to that number of units or micrograms in the tube and no growth as sensitive.

3. Experimental Data:

Four organisms were compared by the above sensitivity procedures and the results are tabulated in Chart II.

DISCUSSION AND CONCLUSION

The need for a disc tube sensitivity method originated with an anaerobic Streptococcus isolated from a deep cyst. The evaluation of this test was undertaken, employing this *Streptococcus, Cl. bifermentans, Cl. capitovale,* and *Erysipelothrix rhusiopathiae.***

* Chloramphenicol and erythromycin crystals were dissolved in a small amount of ethyl alcohol prior to the addition of the broth diluent. Furadantin was obtained as a solution of Polyethylene Glycol 300. Tnia was diluted with broth.

** The latter three micro-organisms were furnished by Miss Minnie Schrieber of the Florida State Board of Health, Jacksonville, Florida.

The results obtained show that when the test organism was found to be sensitive to the tube disc method, it was also sensitive to a low concentration of the drug in the tube dilution procedure. Organisms reported as resistant to the tube disc sensitivity were also resistant, or showed no growth except in relatively high concentrations of the drugs in the tube dilution determination.

From the organisms tested we conclude that this tube disc test gives results comparable to the tube dilution sensitivity procedure. The simplicity of this test makes it easily applicable for use in the routine laboratory.

Chart II
Micro-Organisms

DRUG	Anaerobic Gamma Streptococcus		Clostridium Bifermentans		Clostridium Capitovale		Erysipelothrix Rhusiopathiae	
	Tube Dilution Sens.	Tube Disc. Sens.	Tube Dilution Sens.	Tube Disc. Sens.	Tube Dilution Sens.	Tube Disc. Sens.	Tube Dilution Sens.	Tube Disc Sens.
Penicillin	0.39*	Sens.	0.39	Sens.	0.39	Sens.	0.39	Sens.
Aureomycin	0.39	Sens.	0.39	Sens.	0.39	Sens.	0.39	Sens.
Streptomycin	3.12	Sens.	12.5	Res.	12.5	Res.	6.25	Res.
Chloromycetin	3.12	Sens.	3.12	Sens.	1.56	Sens.	6.25	Sens.
Terramycin	0.39	Sens.	0.39	Sens.	0.39	Sens.	0.78	Sens.
Bacitracin	0.39	Sens.	1.56	Sens.	0.78	Seps.	6.25	Res.
Polymyxin	100	Res.	100	Res.	100	Res.	100	Res.
Erythromycin	0.39	Sens.	0.39	Sens.	0.39	Sens.	0.39	Sens.
Neomycin	12.5	Res.	100	Res.	100	Res.	100	Res.
Tetracycline	0.78	Sens.	0.39	Sens.	0.39	Sens.	0.78	Sens.
Furadantin	50	Res.	50	Res.	25	Res.	12.5	Res.

* Figures listed under tube dilution sensitivity represent the highest dilution of the drug in units or micrograms in which no growth appeared after 24 hours incubation.

Summary

An anaerobic gamma **Streptococcus** was isolated from our patient, Mrs. R. We were unable to read plate disc sensitivities on this organism because it refused to grow readily on solid media. Thus, we were challenged to set up a simple tube procedure for drug sensitivity. A tube disc anaerobic sensitivity test was tried and correlated to the standard tube dilution procedure. Three other micro-organisms were so tested to evaluate the procedure.

With the promising results obtained at this time, it is proposed that this new simplified test be tried as a screening test to drug sensitivity of anaerobic organisms. This test is particularly useful when the cumbersome tube dilution procedure is not in routine use and it is imperative to give the physician a quick result.

Acknowledgements

The author wishes to thank the laboratories of Chas. Pfizer & Co., Eaton, Upjohn, and Eli Lilly for furnishing the drugs used

in this experiment, Dr. J. N. Patterson for his guidance, Doris Dede, M. T. (ASCP), Ann Durgin, M. T. (ASCP) for their technical assistance, and Fannie Sutherland for her assiduity in the typing of this paper.

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4. Eli Lilly Laboratory: "Ilotycin: Directions on Handling the Standard Powder for Sensitivity Tests."

ANNOUNCEMENT

The D. C. Society of Medical Technologists wishes to announce the establishment of the Mary Francis Gridley Loan Fund as a living memorial to our late friend and colleague. This is in accordance with the D. C. Society of Medical Technologists' resolution approved by the House of Delegates of the American Society of Medical Technologists in June, 1955.

We are now soliciting contributions for this fund.

When sufficient money is accumulated loans will be made available to deserving students in American Medical Association approved schools of medical technology and/or registered medical technologists who desire to take advanced or refresher courses.

It is hoped that enough contributions will be received by January 1, 1956 in order to make the first loan available as soon as possible thereafter.

Checks should be made payable to the Mary Francis Gridley Loan Fund and sent to Miss Martha Feldman, M.T. (ASCP), 915 19th Street, N.W., Washington 6, D. C.

FIRST NORTH AMERICAN CONFERENCE OF MEDICAL LABORATORY TECHNOLOGISTS

Doesn't it sound exciting? Need we urge you to write papers to present at this conference? It would be a great HONOR to so participate in this big event. N'est ce pas? Canada and the United States have long been friends. Here is our chance to promote good will and to cooperate with our neighbors!

A NEW AWARD has been added this year—founded in memory of a student Medical Technologist—for STUDENTS ONLY!

Qualified papers submitted, but unable or too brief to be included in the actual program, may be listed in the printed program as "READ BY TITLE."

Mimeographed summaries of as many papers as possible will be available in ENGLISH and FRENCH for distribution.

Approximately $1500 in awards is available to registered Medical Technologists, ASMT members, and student Medical Technologists this year for prize winning papers. The DEADLINE for submitting the TITLE of your paper to the Program Committee is **December 1, 1955.**

Also being offered are eight refresher courses. Among the subjects being included in these courses are chemistry, hematology, bacteriology, histology, serology and blood banking. Each course will offer eight hours of lecture and laboratory demonstrations. This is your chance to "catch up" on the latest technics in the field of your choice.

THE WORK OF THE REGISTRY OF MEDICAL TECHNOLOGISTS

LALL G. MONTGOMERY, M.D.
Chairman, Board of Registry, Muncie, Indiana

It is good to be here and to see so many familiar faces. It was a great disappointment to me to miss last year's meeting, but I had first-hand reports of your excellent program and the fine time you had.

A report from the Board of Registry is not merely a report of what the Registry office staff and the Board have done during the year, but it is in large measure a report of what you have done to assist us in our work. The Board of Registry could not function were it not for the loyal support and assistance furnished by you, the registered medical technologists. And so what I say this morning will be an intermingling of office statistics, Board actions, and how these have been stimulated and accomplished by group action.

As was to be expected, we have grown since last year. See Table 1. Since 1928 we have registered 27,055 medical technologists, including the 572 who passed the May 13 examination. During the past year 528 have been withdrawn for lost address and/or delinquent fees, 30 have resigned, 26 were deceased, and 6 were withdrawn for violation of the Code of Ethics; making a total of 590 withdrawn. There were 71 former registrants reinstated, the largest number we can remember having been reinstated in one year. The number currently registered is 21,199.

Table 1
Number of Registered Medical Technologists

Registered since 1928		27,055
Currently registered		21,199
Withdrawn past year		590
Delinquent fees and/or Lost address	528	
Resigned	30	
Deceased	26	
Violation Code of Ethics	6	
Re-instated		71

Table 2 shows that in other categories there are: 8 Specialists currently registered; 33 Microbiologists; 33 Chemists; 286 Histologic Technicians; 62 Laboratory Aides; and 97 Blood Bank Technologists.

Table 2
Number Registered in Other Categories

Specialists	8
Microbiologists	33
Chemists	33
Histologic Technicians	286
Laboratory Aides	62
Blood Bank Technologists	97

You will remember that we asked on the 1953 questionnaire what you thought should be done about the training of Laboratory Aides. Table 3 shows that of the 10,310 answers to this question, more than half of you thought that there should be no certification of Aides, although on the whole there is no unanimity of opinion as to how these people should be trained, or whether they should be trained at all. No Laboratory Aide applications have been accepted for the past 4 years.

Table 3
Aide Training Should Be:

Discontinued	2,407
On local level, no certification	2,749
Only in Approved Schools, separately	2,291
Only in Approved Schools, with M.T.'s	665
In Aide Schools only	1,972
Other plans suggested	226

As your numbers increase, so does your urge to move or change your name, apparently, because we had a total of 6,824 changes of name and/or address during the year, an average of 569 per month for the year, and during the 5 busy renewal months an average of 889 a month. And because of these changes, 2,702 TECHNICAL BULLETINS were returned to us, an average of 225 a month. The addressograph plate maker was clanging away quite steadily during those months. Some of you changed address more than once, but if each change represented one person, 21 per cent of you changed name and/or address during the year. We might use as our recruiting slogan, "Join the Medical Technologists and get a man and see the world!"

As the profession is publicized and as there are more of you, so there is more mail received in the Registry office—51,857 pieces the past year, exclusive of examination papers, an increase of 2,427 over the previous year. Your tremendous recruitment activities almost doubled the number of letters and cards asking for general information about medical technology; from 2,180 last year to 4,200 this year. Letters which were answered with a marked booklet or a short letter totaled 2,675, an increase of 847 over last year. Requests for supplies of vocational literature increased from 393 last year to 512 this year. As a result of one TV program, we sent out almost 300 packets of High School booklets and copies of the pamphlet "If You Were a Medical Technologist." As a result of a folder prepared by the National Committee for Careers in Medical Technology, we have already sent out 502 packets of material, some containing several of each piece of material, and more requests are coming in every day. The article in CHANGING TIMES, "Jobs Behind the Doctors," brought requests from 89 correspondents who mentioned the name of the article; and the article in TODAY'S HEALTH—86. An article in the January issue of THE CATHOLIC MISS was

also responsible for a number of requests. No doubt many more of the 1100 who asked for general information during the first few weeks of this year read these and other articles but did not mention the source. With these three articles all appearing in January, you can well imagine that we were quite busy mailing vocational materials.

It has been encouraging to note that there has been a considerable increase in the number of transcript evaluations made during the year. There were 1,972 original evaluations made the previous year, and 2,172 this past year. Including copies and revisions there were 3,033 the previous year, and 3,374 this past year. It would seem as if we should have many more applications for the examination next year, if this is an indication of the increase in the number of students in the Approved Schools.

I have already mentioned that we have sent out many more pieces of vocational literature, and of course this is included in the great increase in the number of pieces of outgoing mail. This past year we sent out 86,213 pieces of mail; this included 76,534 first class, as compared with 71,539 last year. The amount of fourth class mail increased from 707 packages of vocational literature to 884 this year. In addition, 74 packages of material were sent by Railway Express.

As we have reported to your Recruitment Chairman, 177,107 pieces of vocational literature were sent in quantity shipments, at a cost of $5,163.08 for the material, a mailing cost of $732.56, making a grand total of $5,895.64, as shown in Table 4. We have not included the cost of the CHANGING TIMES reprints, as these were sent to us by the National Committee for Careers in Medical Technology; nor has the cost of office expenses been included. During the previous year 161,507 pieces of material were shipped in quantities, at a cost of $4,900.11, a shipping cost of $532.95, or a total cost of $5,433.16. During this past year 10,233 pieces of material were used at 11 exhibits, at a total cost of $340.34 for the material. I shall not confuse you further with detailed statistics, but thought you would like to know how we have occupied ourselves during the year, and how your own activities have been reflected in what we do in the Registry office. I assure you that you have kept us hustling.

Table 4
Quantity Shipments of Vocational Materials

Number of pieces	177,107
Cost of materials	$5,163.08
Mailing costs	732.56
Total cost	$5,895.64

On May 13, examinations were given to 725 Medical Technologists, of whom 572 passed. Also given were examinations to

92 Blood Bank Technologists, 6 Chemists, 4 Microbiologists, 2 Microbiologist Specialists, and 61 Histologic Technicians.

You will remember that we gave an examination in Blood Banking last fall for the first time, at the request of the American Association of Blood Banks, and with their complete cooperation. The questions were prepared by the Education Committee of the AABB, and approved by the Board of Registry. There were 110 candidates for the examination, of whom 97 passed and 13 failed. For the practical section of the Fall examination, candidates were assigned by the Standards Committee of the AABB to the examining center nearest to them. This proved to be an undesirable way of handling it, as some candidates were quite far from an examining center, so for the Spring examination blood samples were prepared in a central laboratory and sent Air Mail Special Delivery to each candidate. Instructions for conducting the tests were included, the candidates mailed their results to the Registry office, and we sent them on to a member of the Education Committee of the AABB, where they were scored according to a code decided on by that committee. This plan seems to have worked out well, and was much more convenient for the candidates. The questions have been machine scored and analyzed statistically, as they were the obpective type of questions. A new certificate and membership card were designed for those certified as Blood Bank Technologists as a result of the examination. You will find interesting discussions of the certification of Blood Bank Technologists in the May, 1955, issue of the BULLETIN of the American Association of Blood Banks. It has been a pleasure to work with the American Association of Blood Banks on these examinations. Having attended some of their meetings, I can assure you that they have given serious and sincere consideration to all the many problems concerning certification of qualified Blood Bank Technologists, and have high standards for such certification.

I want to discuss with you some of the things that were brought out in the analysis of the questionnaire sent to all registrants in 1953. On the questionnaire we asked you to indicate your educational status. Table 5 shows what level of education has been obtained by 11,061 of you—over half of the medical technologists currently registered. We are sure that the other half would fall in about the same pattern.

Table 5

Educational Status of Registered Medical Technologists

High school graduation only	314
One year college	167
Two years college	1,571
Three years college	872
Four years college (no degree)	341
Degree in Medical Technology (M.T.)	3,098

Degree other than M.T. major	4,230
Master's degree in M.T.	89
Master's degree other than M.T.	335
Ph.D. degree	21
M. D. degree	23
Part of education after certification	932

Table 6 shows proportions of students passing the examination, who had college degrees. You will see that the figure has been consistently around 65 per cent since we first began to keep such statistics in 1948.

Table 6
Proportion of Candidates Passing Examinations, With Degrees

Fall 1948	62.0%
Fall 1949	67.0%
Fall 1950	68.0%
Fall 1951	67.8%
Fall 1952	67.7%
Fall 1953	65.7%
Fall 1954	66.0%

We asked this question on the questionnaire: "What in your opinion should be the proper minimum requirement for M.T. (ASCP)?" Table 7 shows what 12,606 answered.

Table 7
Suggested Minimum Requirements M.T. (ASCP)

Education	Technical Training	
High school	1 year	43
High school	2 years	139
High school	3-5 years	122
2 years college	1 year	2,099
2 years college	18-24 months	1,329
3 years college	1 year (B.S. in M.T.)	5,138
3 years college	18-24 months	825
4 years college	1 year	2,234
4 years college	18-24 months	303
All other suggestions		374

It is significant that only 2 per cent want high school as the prerequisite to technical training, even though most of these people wanted the technical training period lengthened. Furthermore, only 29 per cent of you are satisfied with the present requirements, but 67 per cent indicate a desire for college requirements in excess of the 2 years now required. We have no way of knowing what ideas these 67 per cent have about what would happen to the 35 per cent of the students now taking the Registry examinations who do not have college degrees. They would be barred from the Approved Schools if only 3-year students were accepted for the examination. Where would they get their training? There are three courses open to them, it would seem—they could go into some other field, they could go on to college and finish the requirements for 3 years of college or a degree, or they could go to an unaccredited school where they would get inadequate training. Which course would they select?

Table 8 shows the number of Approved Schools of Medical Technology presently in operation, and the number of students they can accommodate. More than half of the Approved Schools have affiliations with colleges or universities, and many of these academic institutions offer a degree program whereby students receive a Bachelor of Science in Medical Technology on completion of the technical training. On the other hand, there are some schools who for one reason or another accept only students who have 2 years of college and therefore do not give academic credit. We do not know how many of these schools would have to discontinue their training programs if the 3-year college requirement were put into effect. You have expressed your opinions regarding minimum requirements; the Board of Registry has expressed its opinion. It now remains for the American Society of Clinical Pathologists, for the pathologists who direct the training and work of the medical technologists, to say what they think. This they will do at the meeting of the Society next October.

Table 8

Approved Schools of Medical Technology

Year	Number of Schools	Student Capacity	Number Graduating Previous Year
1936	96	701	No figures
1946	268	1,932	1,125
1951	467	3,348	2,220
1953	538	4,116	1,971
1955	596	4,503	No figures

You will recall the salary studies that have been made periodically since 1941. These have shown a gradual increase in the income of medical technologists over the years. Now the 1953 questionnaire has given us the most complete study ever made of the incomes of medical technologists. For statistical reasons these questionnaire figures probably are not directly comparable with the previous studies, but certain overall comparisons are apparent. In Table 9 you will see that there have been some notable changes.

You will note that in 1950 the largest group of registrants had incomes between $2400 and $3300 annually, but the questionnaire now shows the largest group in the $3300 to $3900 range, which is a $600 overall rise in annual income in 3 years. A still more striking trend is the proportion of registrants with salaries over $3600. In 1950 this was 16.3 per cent of the group studied, while the questionnaire findings show that 43.1 per cent of those replying were receiving over $3600 annual salary.

One more striking change is in the group receiving $4200 and over, which rose from 5.15 per cent in 1950 to 16.03 per cent in 1953.

Table 9
Comparison of Salary Studies—1950 and 1953
Base Salary—No Extras

Salary per Annum	1950 2,547 Registrants Picked at Random	1953 6,724 Registrants from Questionnaire
Less than $1500	.28%	1.77%
$1500— 1800	.43%	.83%
1800— 2100	2.24%	1.2 %
2100— 2400	6.24%	1.5 %
2400— 2700	21.63%	4.8 %
2700— 3000	20.77%	8.46%
3000— 3300	22.38%	20.0 %
3300— 3600	9.7 %	18.22%
3600— 3900	8.0 %	18.05%
3900— 4200	3.18%	9.07%
4200 and up	5.15%	16.03%

These figures all represent national averages, as you know, and there are great local variations which we cannot discuss here.

A while ago I mentioned the amount of material we have sent out in answer to requests for recruitment pamphlets, and you will recall that there were large quantities sent. I feel sure that during the past year recruitment has been a major activity for most of you here today, and many who are not here.

Two of the questions on the questionnaire were designed to give background information and opinions that would be helpful in the recruitment program. One asked how you became interested in medical technology. See Table 10. The other, see Table 11, asked your opinion as to the reason for the shortage of medical technologists.

Table 10
How Interested in Medical Technology

Family physician	867
Wanted to be a doctor	2,651
Friend or relative an M.T.	2,489
High school or college advisor	2,382
Article in paper or magazine	561
Other	2,121

Table 11
Technologist Shortage Due to

Not enough young people	242
Work too hard or confining	1,295
Insufficient recruitment	5,037
Poorly paid	5,949
Other	1,131

The answers to the first question, in Table 10, can be a guide to where we direct our program. They clearly indicate that we need to get to the college and high school advisors with our material. They also indicate that personal contact is important, as 2,489 of you heard about this profession because friends or

relatives were medical technologists. And they indicate that we must get to the family physicians with our information about requirements.

Your answers to the second question, Table 11, can be used by the recruitment committees, as a large number of you think there is insufficient recruitment. Remember, however, that these questionnaires were answered last year and reflect the previous year. Since then the work of the National Committee for Careers in Medical Technology has unified the activities of the medical technologists and pathologists, of the national, state and local groups, and individuals. It has reached out into areas previously untapped or tapped only lightly in passing, and has laid out a broad program which is designed to reach an ever widening public, encompassing professional and educational organizations, lay groups, and individuals. No doubt if a questionnaire were circularized next year, the answer to the question as to why there is a shortage of medical technologists would be quite different. Certainly the long-range program of this committee will enable you to point with pride to the quantity and quality of recruitment of medical technologists. However, the National Committee for Careers in Medical Technology is YOUR COMMITTEE, and without you there would be no committee. The Executive Secretary, Miss Dallas Johnson, the representatives from the American Society of Medical Technologists, the American Society of Clinical Pathologists and the College of American Pathologists could not function were it not for your constant cooperation. They can lay the foundation, put up the framework, but you have to finish the job.

Miss Johnson has discussed some of the committee's activities and plans elsewhere, but I want to mention a few things here. First, of course, is the film, CAREER: MEDICAL TECHNOLOGIST, which had such a successful premiere at the International Congress of Clinical Pathology in Washington, D. C., on September 6, 1954. Since then it has been shown many times, at many kinds of meetings, and on more than half the television stations in the country. Reviews of the film have appeared in numerous journals. There has been nothing but praise of the excellence of the film; in fact there are several experts in professional fields who have said it is the best film of its kind they have ever seen. Here is one recruitment tool that sells itself and sells your profession, in a graphic way. It must and will have even wider distribution.

Another excellent tool made available to guidance counsellors this year is the HEALTH CAREERS GUIDEBOOK, published by the National Health Council and supported by the Equitable Life Assurance Society of the United States. This guidebook is as good as anything we have seen along this line. It has been

distributed to the guidance people in the high schools and secondary schools and junior colleges, and copies have been sent or will soon be sent to key recruitment workers in your organization, to your Executive Secretary, to the Executive Secretary of the American Society of Clinical Pathologists, and to the National Committee for Careers in Medical Technology. Before this was ready for publication, we had the opportunity to send material, statistics and information for the section on Medical Technology and certain related fields. Mrs. Drummond worked right with the writers in New York, going over the copy word by word, until everyone concerned was satisfied that these sections were accurate and presented the necessary information completely. We were grateful to have had an opportunity to assist with this project.

I have mentioned the response we received from the articles in CHANGING TIMES, TODAY'S HEALTH, and THE CATHOLIC MISS. There were also articles in the AMERICAN OBSERVER, mention of the film and the need for medical technologists and of the Registry requirements in several other publications, such as U. S. NEWS AND WORLD REPORT, THE LADIES' HOME JOURNAL, THE COSMOPOLITAN, MADEMOISELLE, and GLAMOUR. There have been numerous feature stories in your local newspapers about medical technology, Approved Schools and pathologists, all of which serve to educate the lay public about your profession and the place it occupies in the medical field. Miss Johnson will discuss these things with you in greater detail in the Recruitment and Public Relations Workshop, and Miss Audrey Murphy will give you other interesting and helpful advice and information.

Another excellent booklet made available this past year is "Medical Technologists and Laboratory Technicians," Medical Series Bulletin No. 203-4, published by the U. S. Department of Labor. It was prepared by Miss Mildred Barber under the supervision of Miss Lillian V. Inke, and we were happy to have had an opportunity to read it in manuscript and to offer suggestions for the very few changes that seemed advisable. If you have not seen this pamphlet, you will want to order one from the Superintendent of Documents, U. S. Government Printing Office, in Washington.

I have given you a brief picture of the past year's activities. What of the future? Is it uncertain? On one hand no, because it has been charted, and that is a long step toward a certain future. On the other hand yes, slightly, because in these days of change and because of the inherent frailties of human nature, anything might happen that would blow us off the course. Even so, the future is not dark, and so long as there are loyal medical technologists who know the part they play in that

future, and who have a broad vision of what will be best for those who follow in their footsteps, the future is bright. The future of medical technology depends to a great extent on our present attitudes, our concept of what will be best for the greatest number of people, our realization that of foremost importance is the patient and his welfare. All of our plans for building the future of medical technology must be tailored to fit that ideal.

And now in closing I would like to pay tribute to a medical technologist who has done much for you, for medical technology, for pathology, for medicine. Doctor Emma Moss, Chief Pathologist at Charity Hospital here in New Orleans, is more than just head of the Department of Pathology at the largest hospital of its kind in the world. She personifies Medical Technology and Pathology at their best. She was once a medical technologist, went into medicine, became a pathologist, has inspired and taught hundreds of medical technologists and pathologists, has won recognition for herself and her associates in her particular fields of parasitology and mycology, and she is President-Elect of the American Society of Clinical Pathologists, the first woman ever to be so honored. She is your good friend and ours. With the future in the hands of a friend so devoted to the cause of medical technology, we can proceed to do our part with renewed hope and enthusiasm.

LABORATORY AIDS IN THE DIAGNOSIS OF LEPROSY*

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Leprosy is a chronic communicable disease of Man. Although its path through various countries of the world may be traced through many centuries and although numerous workers have devoted long years to the study of it, leprosy may well be among the last of the chronic infectious diseases to reveal the more important bits of essential basic information.

While it is commonly believed that leprosy occurs rarely or not at all in our present-day world, the best estimates of its current world incidence suggest a figure of five million cases. The United States is one of several countries which enjoy a very low incidence rate. Experienced workers in this country believe that there are perhaps fifteen hundred cases at the present time. During the last twenty years the population of the National Leprosarium at Carville has varied between 300 and 400 patients. The illness is often found in several generations of the same family group, but it is the consensus of epidemiologists that such a pattern results from household exposure to a communicable disease rather than from any hereditary factor.

In the United States there are certain endemic areas in which the disease is known to propagate itself; namely, Florida, Louisiana, Texas, and, to a lesser extent, certain areas of California. The following is a summary of first admissions to the National Leprosarium, grouped according to place of residence at time of admission, from 1894 through 1954:

First Admissions to National Leprosarium 1894-1954

Louisiana	734
Texas	357
Florida	117
California	299
All other States	402
Total First Admissions	1909

The causative agent of leprosy is the Mycobacterium leprae, first seen by the Norwegian physician, Gerhard Armauer Hansen, in 1868 and first described in his writings of 1874. The organism is an acid-fast bacillus not distinguishable morphologically from M. tuberculosis. The latter organism may of course be differentiated by culture and animal inoculation studies. Although M. leprae was among the first bacteria studied in the earliest days of bacteriology some eighty years ago, it has, up to the present day, successfully defied the many thousands of attempts to cultivate it outside of the human body. Neither has it been possible

* Read before ASMT, June, 1955, New Orleans, La.

to reproduce the disease in laboratory animals by the introduction of bacilli laden human material. Thus, at the very threshold of studying the disease, one is confronted by major obstacles which seriously hamper investigations in epidemiology, pathogenesis, pathology, therapy and other fields. Although Koch's postulates have never been fulfilled, observations of the past eighty years have led all to accept M. leprae as the etiologic agent.

It is essential that brief mention be made of the classification of leprosy since the clinical picture of a single case may often represent so small a piece of the total mosaic pattern as to be scarcely recognizable. Two polar extreme types of leprosy are generally described:

1. The lepromatous (or nodular) type shows little or no resistance on the part of the host to the presence or multiplication of bacilli, which are found in unbelievably large numbers in all active lesions. The lepromatous type may be suggested by its typical attack of the skin with a wide variety of lesions, the mucous membrane of the eye and upper respiratory tract, the peripheral nerves and often other organs. There is generally no reaction to the lepromin test. Biopsy sections show typical histopathologic architecture of a granulomatous nature studded with macrophages and histiocytes and containing abundant bacilli. Untreated lepromatous leprosy follows a chronic downhill course, and death usually results from a complicating amyloid disease or some intercurrent infection.
2. The tuberculoid (or neural) type indicates a high level of host resistance to the invading organism. It is often difficult or impossible to demonstrate M. leprae in tuberculoid lesions except during rather brief periods of acute reactional states. The clinical picture is usually one of asymmetric distribution. Peripheral nerve involvement is common while skin lesions are often insignificant or absent. A positive reaction to lepromin is generally found in tuberculoid leprosy. The histopathologic picture is likely to resemble sarcoid lesions in which many lymphocytes, epithelioid cells and giant cells are found. Tuberculoid leprosy tends to be extremely stable with long periods of quiescence which may be punctuated by infrequent acute reactions. Although prognosis for life is good, many patients are totally disabled by longstanding neural involvement.

The classification of leprosy also includes an indeterminate group of cases which do not meet the criteria of either of the above polar types. As a group they represent less stable infections with variable outcomes; occasionally they tend later to become lepromatous in type while more rarely there is a tendency

toward the tuberculoid type.

Diagnosis of early leprosy is an all important factor in the adequate management of the individual patient and in any program directed toward the public health control of communicable disease. The far advanced textbook example of leprosy is easily recognized by the untrained eye; the more bizarre clinical picture often presented in earlier stages of leprosy may prove puzzling to the well trained doctor. Since the disease is uncommon in this country, the diagnosis may at times be missed because leprosy is not included among the diseases considered in differential diagnosis. Although the diagnosis of disease is the responsibility of the physician, it is recognized that he is, in most instances, only a member of the diagnostic team and that he must often rely upon laboratory procedures for confirmation of diagnosis. It is to be hoped that the diagnostic acumen of a large number of practicing physicians may be sharpened by mention of a few laboratory tests which are helpful in the diagnosis of leprosy.

Since the diagnosis of this particular clinical entity carries with it a great weight of psychic trauma, it should be reached only after conclusive confirmatory tests have been made. The best proof of the presence of the disease is the finding of M. leprae in deep skin scrapings. It is essential that the site of the scraping be chosen carefully to avoid confusion with other acid-fast bacilli which may be found on the skin surface, about the nails, on the beds of trophic ulcers, in smegma or in some other situations. In lepromatous leprosy M. leprae may be found easily in nodules, plaques, papules, macules or areas of diffuse skin infiltration. In the absence of discrete lesions specimens may be obtained from the supraorbital ridges, the earlobes, the elbows and the buttocks, since they are sites of predilection and may often reveal a small number of organisms in the absence of discernible skin lesions. The site selected for the scraping is cleansed with alcohol, dried and pinched firmly between the examiner's thumb and forefinger to effect blanching of the part; an incision 4 or 5 mm in length and 2 or 3 mm in depth is made in the blanched area; the knife or razor blade held at right angles to the line of incision is then scraped rather vigorously through the small wound until a small specimen of tissue juice and cellular debris is obtained on the tip of the blade. The specimen is transferred to a clean glass slide, air dried, and fixed by flaming briefly. The specimen thus obtained is stained by the Ziehl-Neelsen or other suitable acid-fast technic, and a thorough search for acid-fast bacilli is made.

Untreated lepromatous lesions will reveal myriads of organisms; specimens obtained after many years of sulfone therapy may show very few organisms, scattered acid-fast granules or nothing at all. Repeated examinations of patients with tuberculoid leprosy may fail to reveal any acid-fast bacilli unless the

patients are in an acute reactive phase when a few scattered M. leprae may be found after diligent search.

It is true that M. leprae may also be found in scrapings of the nasal mucosa in active lepromatous leprosy. It is emphasized, however, that the diagnosis of leprosy should not rest solely upon the results of nasal scraping examination. M. tuberculosis may occasionally be found in the nose of a patient with tuberculosis. Saprophytic acid-fast bacilli found on the nasal mucosa may be confused with M. leprae. Extensive surveys indicate that the nasal mucosa seldom harbors M. leprae in patients whose skin scrapings fail to demonstrate the organism. If a nasal scraping is indicated as a guide to the prognosis of a patient during treatment, the specimen may be obtained from an ulcerated area of the nasal mucosa, or, if the nasal mucosa is intact, it may be incised and scraped gently with a sharp instrument.

The histopathologic study of biopsy specimens is an additional aid in the laboratory diagnosis and classification of leprosy. Pathologists recommend that biopsy specimens be of sufficient depth to include a part of the subcutaneous adipose tissue. Sections for histologic study are usually stained with hematoxylin and eosin. Comparable tissue sections should also be stained by an acid-fast technic and a search made for M. leprae. Unless the tissue contains a very large number of M. leprae usual methods of handling paraffinized sections may give disappointing results. Superior results are obtained if one follows the simple procedure described by Fite, Cambre and Turner[1] in which the sections are de-paraffinized by two washings in a mixture containing two parts of xylene and one part of a light oil (cottonseed, peanut or olive oils are satisfactory).

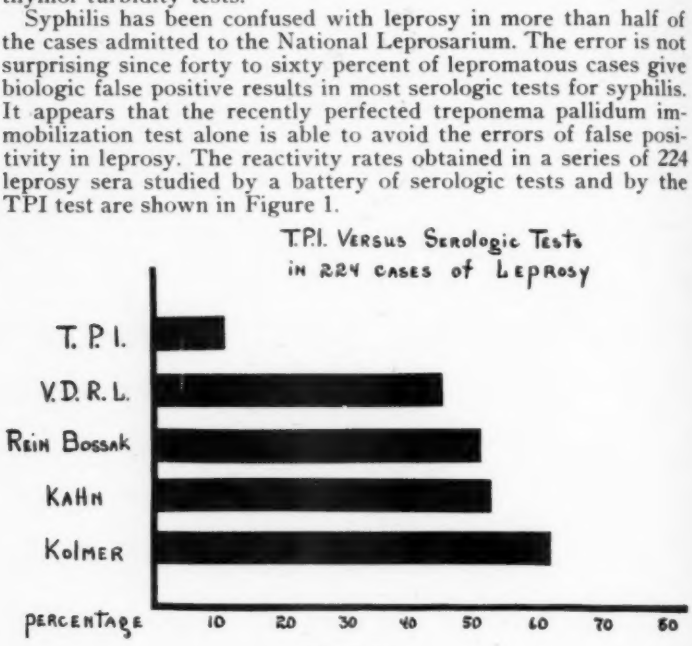
There are certain cases of leprosy, notably the tuberculoid and the longstanding polyneuritic type of lepromatous disease, in which skin lesions are insignificant or absent. In such cases the demonstration of M. leprae or typical histopathologic architecture may be difficult or impossible by usual examination. To avoid the diagnosis of leprosy on a strictly clinical basis it may be desirable to employ a concentration technic on a small biopsy specimen obtained from any part of the skin surface. A satisfactory method of concentration which includes acetic acid digestion and mechanical homogenization was described by Khanolkar[2] in 1952; by this method of examination acid-fast bacilli may be demonstrated in practically all cases of leprosy.

Since there is some confusion concerning the potentialities of the lepromin test, it may be mentioned here that the test is definitely not a diagnostic one. It has been shown that both positive and negative results may be obtained in people who do not have leprosy. The test is helpful in classifying an already diagnosed case of leprosy. It is performed by the intradermal injection of

0.1 cc of lepromin antigen which is a saline suspension of killed lepra bacilli obtained from a lepromatous lesion. The appearance of an inflammatory papule three weeks after injection is considered a positive response and occurs generally in tuberculoid leprosy. In lepromatous disease there is usually no response.

Blood chemistry studies may also be helpful in the classification of leprosy. Well established cases of lepromatous disease usually show a reversal of the A-G ratio due to hyperglobulinemia. The abnormality of serum proteins is associated in many instances with positive results in the cephalin flocculation and thymol turbidity tests.

Syphilis has been confused with leprosy in more than half of the cases admitted to the National Leprosarium. The error is not surprising since forty to sixty percent of lepromatous cases give biologic false positive results in most serologic tests for syphilis. It appears that the recently perfected treponema pallidum immobilization test alone is able to avoid the errors of false positivity in leprosy. The reactivity rates obtained in a series of 224 leprosy sera studied by a battery of serologic tests and by the TPI test are shown in Figure 1.



Conclusion

There is a particular need for prompt diagnosis of leprosy in this country where the disease is uncommon with a total incidence of approximately fifteen hundred cases. Attention is called to the fact that certain laboratory procedures may be helpful, if not essential, in furnishing confirmatory proof of diagnosis or guideposts in the classification and prognosis of the disease.

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DETERMINATION OF SERUM CALCIUM BY A CHELATING METHOD USING A SPECTROPHOTOMETER†

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The methods commonly used for the determination of serum calcium levels in the clinical laboratory are either lengthy, requiring at least two hours for the Clark and Collip[3] modification of the Kramer and Tisdall[6] method, or lack reproducibility, especially with the Roe and Kahn[8] method. Methods using the flame photometer[7,9,11] have been advocated but these either have not been very successful or accepted by most laboratories. Recently methods using the chelating agent, disodium dihydrogen ethylenediaminetetraacetate (EDTA), have been proposed.[1,4,10] Many of these latter procedures use a visual endpoint which is difficult to determine. Fales[4] has adapted this method for both a micro and macro procedure using a spectrophotometer to determine the endpoint. By using ammonium purpurate as the indicator, the color change is so gradual that a spectrophotometer is necessary to obtain accurate results.

The following macroprocedure, which is essentially that as outlined by Fales[4,5] has been found to be satisfactory for use in the clinical laboratory. An important modification is the determination of the optimum concentration of indicator, which was found independently by Fales[5] and by us. If the diluted indicator is too concentrated, the endpoint is masked even with the use of a spectrophotometer. Therefore, it must be diluted to a predetermined optimum range of transmittency.

METHOD

Reagents

1. 9 Normal sodium hydroxide.
2. Ammonium purpurate indicator. To 30 ml. of distilled water add the contents of 1 capsule‡ (0.25 gm.). This solution should be stored in a dark bottle and kept in the refrigerator. It should keep for about 1 month and be thoroughly shaken before use.
3. Standard disodium dihydrogen ethylenediaminetetraacetate. Weigh out 0.18 gm. of EDTA§ and dissolve it in 200 to 300 ml. of distilled water in a liter flask. When dissolved,

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‡ The capsules of ammonium purpurate were purchased from the Hagen Corp., P. O. Box 1346, Pittsburgh 30, Pa.
§ We used Sequestrene Na_2 purchased from the Alrose Chemical Co., Providence 1, R. I. The Bersworth Chemical Co., Framingham, Mass., produces the same product under the trade name Versene.

dilute to volume. One ml. of this standard is equivalent to approximately 0.02 mg. of calcium. Store in a polyethylene bottle.

4. Standard calcium solution. Oven-dry, reagent grade, calcium carbonate. Weigh out 2.497 gm. of the dry material into an evaporating dish. Dissolve the carbonate in a minimum amount of concentrated HCl and evaporate to dryness in a hood. Wash the resulting calcium chloride into a 100 ml. volumetric flask and make to volume with distilled water. To prepare the working standard, dilute 10 ml. of this stock to 1 liter with distilled water. The working standard contains 10 mg. of calcium per 100 ml.

Equipment

1. Coleman Junior Spectrophotometer.
2. Coleman Cuvets, 19 by 105 mm.
3. Calibrated serological pipet which can be stored in a large test tube fastened to the side of the bottle containing the EDTA solution. Calibrate this pipet by titrating triplicate solutions of standard calcium solution and then calculate a factor for the pipet by the method which follows. For example, if the calcium standard (10 mg. per 100 ml. solution) requires 5.2 ml. of standard EDTA solution for the titration, then the factor for the pipet is 1.92 (calcium equivalence of 1 ml. of EDTA solution). A new factor must be determined for each new batch of EDTA solution and for each change of pipets. The pipet is to be blown out at the end of each delivery, and it is suggested the same pipet be retained for all calcium determinations. If an automatic buret is used, a standard should be run with each set of determinations.

Procedure

1. Use one cuvet for each unknown serum or standard to be run and add one for the blank. Five determinations can be run with each blank.
2. Prepare the indicator solution in a beaker by adding 10 ml. of distilled water for each determination to be run, including the blank. For each 10 ml. of water add 0.2 ml. of 9 N NaOH. Add 3 drops of ammonium purpurate indicator and mix. Place 9 ml. of this mixture in a cuvet and read in the spectrophotometer at 620 mu. against a water blank set at 100 per cent transmittency (%T). Continue adding indicator dropwise until the solution in the beaker records 25 to 35 %T. If the mixture reads less than 25 %T, add 10 ml. of water and 0.2 ml. of 9 N NaOH to dilute it.

3. Place 1 ml. of unknown serum in a cuvet and if a standard is being run, place 1 ml. of calcium standard in another tube.
4. To each cuvet, including blank, add 9 ml. of the diluted ammonium purpurate indicator.
5. To the blank add 2 ml. of the EDTA solution from the calibrated, blow-out pipet, and to the other cuvets, add 1 ml. of EDTA.
6. Mix the materials thoroughly. (Finger cots which can be wiped dry after each inversion of the cuvets reduce the loss or transfer of the solution and keep the outside of the cuvets from becoming wet.)
7. Set the blank at 30 %T on the Coleman at 620 mu.
8. Readings of each unknown or standard solution are then taken with the blank being reset at 30 %T before each reading to compensate for fading of the indicator when exposed to light.
9. After taking the first set of readings, 1 ml. of EDTA is again added to each tube, including the blank. Mix thoroughly.
10. Readings are again taken with the blank reset at 30 %T and balanced before each reading.
11. Continue adding 1 ml. amounts of EDTA and take readings after each addition until there is no further decrease in transmission.
12. The endpoint is determined by plotting on semilogarithmic paper the % transmittency values along the ordinate against the ml. of EDTA used along the abscissa. The endpoint is the intersection of the lines formed by the steepest portion of the titration curve and the plateau formed by the points showing no further decrease in %T.

Calculation:

$$\text{mg. calcium/100 ml.} = \frac{\text{endpoint (graph)}}{\text{ml of EDTA used}} \times \text{pipet calibration factor}$$

Fales has emphasized the following precautions:

1. Make readings immediately after the conclusion of the swing of the galvanometer; then insert the blank in the well and record the previous reading while the galvanometer is becoming stabilized.
2. Do not leave tubes in the Coleman spectrophotometer for any extended period of time since this increases the fading of the indicator.
3. Hemolyzed blood is unsatisfactory for calcium determinations inasmuch as the ionic iron interferes with titration as its concentration increases.

As far as is known at this time, an icteric serum does not affect the calcium titration as long as the icterus does not mask an accompanying traumatic hemolysis. However, this must be verified by further determinations.

Results and Discussion

Duplicate serum calcium determinations as performed in the clinical laboratory using both the EDTA and the Kramer-Tisdall methods are presented in Table 1. It is seen that values of serum calciums determined by EDTA titrations are not only reproducible, but also compare with results obtained on duplicate specimens with the Kramer-Tisdall procedure. Also the recovery of added calcium to serum specimens has been satisfactory.

Table 1

Values of Serum Calcium (in mg. per 100 ml. serum) Obtained by Two Methods and Performed in Duplicate

No. of Patient	Kramer-Tisdall* Method		EDTA Method		Recovery† EDTA Method	
1	10.6	10.3	10.5	10.5	15.4	15.4
2	10.7	10.7	10.7	10.7	15.7	15.7
3	10.5	10.7	10.5	10.5	15.4	15.4
4	9.6	9.7	9.6	9.6	14.6	14.6
5	9.6	9.6	9.6	9.6	14.6	14.6
6	9.5	9.5	9.4	9.4	14.6	14.6
7	10.0	10.0	9.7	9.7	14.7	....
8	9.7	10.0	10.0	....	14.8	....
9	9.9	9.9	9.6	9.6	14.7	14.7
10	9.9	10.0	10.4	....	15.4	....

* Modification of Clark and Collip.
† 5 mg. of calcium was added to each 100 ml. serum.

Using ammonium purpurate as an indicator, a red color appears in the presence of ionic calcium, and gradually changes to a purple color as the calcium is deactivated or deionized by the EDTA. Fales[4] points out that this indicator is specific for calcium at a pH over 12. Buckley and Gibson[2] suggest using "to deliver" pipets to avoid introducing exhaled carbon dioxide and subsequently lowering the pH. However, using "blow-out" pipets, we found the pH of the solutions 12.4 both before and after titration, and also no apparent effect on the pH from the buffering action of the serum. This method gives total serum calcium (ionic plus protein-bound) levels as does the Clark-Collip procedure with comparable results being obtained in both low and elevated serum protein levels by the two methods.[4]

The principal advantage of this procedure over those commonly employed for the determination of serum calciums is reproducibility or precision. In addition, the time required is less than that of other methods, and the entire procedure can usually be completed in 20 minutes. Duplicate determinations can be performed on the amount of serum normally required for a single test using the oxalate precipitation-permanganate titration method. After the EDTA has been standardized, the only solution that is unstable and must be replaced monthly is the

indicator.

One disadvantage of this procedure is that a spectrophotometer must be used and the attention of the technologist must be given to it for a period of about 15 minutes. Also each determination must be plotted on graph paper which, however, does not deter from the procedure after the technologist has had the experience of plotting the values from several titrations.

Summary

A chelating method for the determination of serum calcium is presented. This utilizes the spectrophotometer for the determination of the endpoint and is essentially the same as proposed by Fales with several modifications. The method is comparatively rapid and simple as well as accurate and is suitable for use in the clinical laboratory.

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MODERN METHODS IN DIAGNOSTIC BACTERIOLOGY*†

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In recent years marked progress has been made in the laboratory diagnosis of bacterial infections. Not only has our knowledge of pathogenic bacteria increased, but also our methods of handling these organisms has improved. The advances have been accomplished largely through the improvement of bacteriological media. The way of medical bacteriologists has been made much easier and their results more accurate by the development of media to solve specific problems of isolation and identification. Aid has also been contributed by the serologists, whose elucidation of the antigenic structure of bacteria has led to the development of commercially available antisera for the rapid selection of certain genera and species.

Other factors have influenced the development of today's procedures. The great increase in the number of bacteriologic examinations requested by the clinical staff has necessitated the simplification of methods so that a large number of specimens can be handled quickly and efficiently with results that are accurate and, what is most important, clinically significant. The introduction of chemotherapy and antibiotics has also had a marked effect. To be helpful to the clinician results must be obtained and reliable reports made available as rapidly as possible. Therefore, time consuming procedures have been discarded and suitable short-cuts adopted whenever possible. Modern therapy has also changed the emphasis in diagnostic bacteriology in many cases. Procedures which were once of clinical significance are now of academic interest only. Others, particularly those involved in the rapid recognition and identification of antibiotic resistant organisms, have assumed major importance.

It is the purpose of this paper to review briefly some of the improved procedures available today and to stress important points and pitfalls which may be overlooked. Detailed methods will not be given, since these are available elsewhere,[1] but what is intended is rather a broad survey of the most common problems in modern diagnostic bacteriology.

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I. ENTEROBACTERIACEAE

Consideration will first be given the methods used in dealing with the Gram negative bacilli of the family *Entrobacteriaceae*. It is in this field that most progress has been made in the past twenty-five years. Improvements in isolation media, leading to the wide spread use of SS agar, desoxycholate agar and/or MacConkey agar, and the introduction of enrichment media such as selenite broth, have greatly increased the number of isolations of the enteric Gram negative bacilli, and there is no longer any great problem involved in the isolation of pathogens from stool specimens. The identification of the enteric pathogens, *Salmonella* and *Shigella*, and the differentiation of these pathogens from Gram negative bacilli not pathogenic in the intestines, have been greatly facilitated by the introduction and general acceptance of multiple sugar media such as triple-sugar-iron agar (T.S.I.), which in twenty-four hours will determine the fermentation of detrose, lactose and/or sucrose and the production of hydrogen sulfide. More complete knowledge of the serology and serologic inter-relationships of this group has led to the commercial production of dependable antisera for the selection of *Salmonella typhi*, other *Salmonella* and *Shigella*. The selection of these pathogens by their reaction on T.S.I. agar and their serologic reaction with commercially available antisera in the rapid slide agglutination test, and the shipment of the culture to the local State Health Department for complete antigenic analysis and definite identification is certainly a simple solution to what used to be a very big problem.

However, it should be emphasized that in this system of rapid selection of enteric pathogens there are certain defiinite sources of error. It is fairly common experience to send a culture for *Salmonella* typing only to have it reported back as a paracolon bacillus or possibly even a *Proteus* species. Salomonella Typing Centers are overloaded with such organisms which have been erroneously identified by clinical laboratories. It is not possible to rely entirely on the selection of enteric pathogens on the basis of T.S.I. reactions and slide agglutination tests. Ewing,[2] of the Communicable Disease Center, emphasizes this point in a recent publication. He points out that many cross reactions can and do occur. For example, *Shigella* and coliform bacilli share a number of "O" antigens, and an anaerogenic (non-gas forming) paracolon bacillus which reacts like *Shigella* on T.S.I. agar may also agglutinate with *Shigella* antiserum. *Proteus* and paracolon strains frequently agglutinate with polyvalent *Salmonella* antiserum and occasionally with typhoid-Vi and flagellar-d. Ewing urges that bacteriologists determine biochemical reactions beyond the preliminary test on T.S.I. agar before jumping to conclusions on the basis of positive

slide agglutination tests with Shigella or Salmonella antisera.

What biochemical tests are most useful in confirming the identity of an enteric pathogen? It is usually preferable to choose one or two tests which will confirm or disprove the tentative diagnosis, rather than to run through a whole battery of tests. Very useful is the urease test medium of Rustigian and Stuart,[3] which allows the rapid identification of organisms of the *Proteus* genus. It is advantageous to inoculate this urea broth at the same time T.S.I. slant is inoculated. In this manner *Proteus* species can be recognized in twenty-four hours with the saving of time and trouble formerly spent in detailed biochemical studies. In this connection, a modified method of making up urea broth should be mentioned, since it works well and is very simple. The directions for preparing urea broth from dehydrated product require that it be sterilized by filtration, which is troublesome and not always succesful. A much simpler method of sterilization has been suggested by Rhodes,[4] in which the medium is made up from the dehydrated product in twenty times its normal strength and placed in a flask with a few crystals of thymol. The flask is sealed with a sterile rubber stopper and stored at room temperature. In twenty-four hours this concentrated medium is sterile and can be stored in this form indefinitely. To make up the urea broth for use, the concentrated medium is diluted twenty times with sterile distilled water, using aseptic technique. The diluted medium is then distributed aseptically into sterile Wassermann tubes in 2 ml. quantities. This chemically sterilized urea broth will support the growth of appropriate organisms and gives reliable results. The small laboratory may also find it convenient to use thymol sterilization for the preparation of media to determine sugar fermentations. Ten per cent aqueous carbohydrate solutions can be chemically sterilized and stored in screw cap tubes. Basic media containing an indicator, such as Difco purple broth base or B.B.L. phenol red broth base or C.T.A. medium, can be kept on hand. When fermentation media are required, simply add 0.5 ml. of the thymol-sterilized sugar solution to 5 ml. of the basic medium. This method has the added advantage of avoiding heat sterilization which may result in the break down of some carbohydrates.

The most troublesome organisms which must be differentiated from the enteric pathogenic Gram negative bacilli are the paracolon bacilli, which can resemble *Shigella* and *Salmonella* biochemically and serologically. All medical bacteriologists must be completely familiar with the characteristics of these organisms, which are so common in urinary tract infections, in stool specimens, and which may occur in other clinical material. First, it is necessary to understand and to be able to recognize those paracolon bacilli which are late-lactose fermenting or lactose negative

variants of *Escherichia, Aerobacter,* or intermediate coliform bacilli. For these the generic classification *Paracolobactrum* has been suggested. Biochemically, they most commonly resemble hydrogen sulfide-negative *Salmonella,* while the less common anaerogenic forms resemble *Shigella.* As previously mentioned, due to common "O" antigens, they may agglutinate with *Salmonella* or *Shigella* antisera. A complete biochemical study of these paracolon bacilli, requiring ten to fifteen different media, is impractical, considering the large number isolated from clinical material. It is necessary to use short-cuts. For example, indol produciton by coli-variants *(Paracolobactrum coliforme)* differentiates these paracolon bacilli from Salmonella, which are uniformly indol-negative. These coli-variants are the most common of the paracolon bacilli and are easily identified by this one test. Urease production by most of the aerobacter-variants *(Paracolobactrum aerogenoides)* differentiates these paracolon bacilli from *Salmonella* and *Shigella.* For detection of urease production by these organisms it is necessary to use Christensen's urea agar,[5] since Rustigian and Stuart's urea broth demonstrates urease production by *Proteus* species only. Like urea broth, this urea agar can be prepared from the dehydrated product by thymol-sterilization, eliminating filtration. The concentrated medium, sterilized with thymol as described above in the preparation of urea broth, is added in an appropriate amount to a sterile 1.5-2% agar and water base. It is then tubed aseptically in Wassermann tubes and slanted so as to produce a butt approximately ¾ inch.

Another simple method of recognizing paracolon variants of *Escherichia* and *Aerobacter* is that described some years ago by Schaub.[6] For this test a diluted (one loop in a tube of sterile broth) suspension of the organisms is necessary, and for this dilution a freshly inoculated tube of indol broth prepared for the determination of indol production (see above) can be used, thus eliminating an extra step. If this diluted suspension of Gram negative bacilli is streaked on SS agar and desoxycholate agar, these paracolon bacilli will show good growth on desoxycholate agar, which serves as a control, but will be inhibited on SS agar. This is only to be expected, since if *Escherichia coli* is inhibited on SS agar, a paracolon bacillus derived from *E. coli* should also be inhibited. The inhibition is not always complete. Occasionally only the size or number of the colonies is decreased as compared to the desoxycholate control. Inhibition is better with coli-variants than with aerobacter-variants. However, it is a helpful test and a simple method of eliminating *Salmonella* and *Shigella,* which grow well on SS agar.

Another group of paracolon bacilli which it is important to be able to recognize are the so-called Bethesdas, which were first described by Barnes and Cherry[7] from an outbreak of gastro-

enteritis at the Naval Hospital at Bethesda, Maryland. These are sometime classified as *Paracolobactrum intermedium* on the basis of their IMVIC formula, but they are quite different from other *P. intermedium* strains, which are late-lactose fermenting variants of typical intermediate coliform bacilli, and which belong to the group just discussed which are inhibited on SS agar. The Bethesdas ferment lactose slowly, requiring from two days to two weeks, produce acid and gas in dextrose, abundant hydrogen sulfide, and are not inhibited on SS agar. Most important, they regularly agglutinate with polyvalent *Salmonella* antiserum. Therefore, on SS agar, T.S.I. slant and slide agglutination test they are identical with hydrogen sulfide-producing *Salmonella*. For their identification a special antiserum is available from the Diagnostic Serum Fund, 2236 Highland Avenue, Birmingham 5, Alabama. All hydrogen sulfide-positive organisms suspected of being *Salmonella* should be tested serologically with both *Salmonella* and this special paracolon antisera. As an additional check, a lactose fermentation tube can be inoculated and held for at least two weeks before discarding as negative. In this way confusion of Bethesdas and *Salmonella* will be avoided.

The last of the important paracolon bacilli is that designated as 29911 by Stuart, Wheeler and McGann[8, 9] and also known as the Providence group because of Stuart's association with Brown University at Providence, Rhode Island. Paracolon 29911 is lactose negative. The majority of strains ferment dextrose with a very small amount of gas, but some strains are completely anaerogenic. On T.S.I. agar Providence paracolons resemble *Shigella*. Their biochemical differentiation is very simple, however, since 29911 is motile and citrate positive while *Shigella* species are non-motile and do not utilize citrate. If the rule is followed that no organism be reported as *Shigella* species unless it is proven to be non-motile and citrate negative, there will be no confusion of paracolon 29911 with Shigella.

The present status of Friedlander's bacillus, or more correctly *Klebsiella pneumoniae*, represents a definite clarification of an old problem. It has been evident to many bacteriologists for years that there is no such specific organism as Friedlander's bacillus, and that this encapsulated Gram negative bacillus, the etiological agent of a specific type of lobar pneumonia, cannot be differentiated from encapsulated Gram negative bacilli occurring in other clinical material. Edwards[10] as early as 1929 and Osterman and Rettger[11] in 1941 concluded that there are no valid criteria for the differentiation of *Klebsiella pneumoniae, Aerobacter aerogenes* or *Paracolobactrum aerogenoides*. This opinion was definitely confirmed in 1952 by Edwards and Fife[12] who state, "No basis for the separation of the two *(Klebsiella* and *Aerobacter)*, either upon type of disease produced or upon biological characteristics, is known."

They also report finding 57 serologic types of encapsulated Gram negative bacilli, thus demonstrating the futility of the attempts of the clinical bacteriology laboratory to identify *Klebsiella pneumoniae* on the basis of quelling reactions with the two types of antisera which have been commercially available in recent years. Edwards and Fife conclude that the *Klebsiella* and *Aerobacter* genera should be combined and recommend classification on the basis of serology with practical identification resting upon recognition of capsule types.

Problems still remain in the differentiation of certain Gram negative bacilli which resemble the *Enterobacteriaceae* in growth and biochemical characteristics. These troublesome organisms are those which give a negative reaction (alkaline slant and butt) on T.S.I. agar, and which may fall into the *Alkaligenes, Achromobacter, Pseudomonas, Flavorbacterium* or other genera. One type has recently been sorted out of this confused group; that is *Bacterium anitratum*,[13,14] which has also been designated B5W by Stuart.[15] There is still a difference of opinion as to the taxonomic position of this organism or group of organisms. It has been suggested by Ewing[16] that *Bacterium anitratum* belongs to the Tribe *Mimeae* of DeBord. This is difficult to confirm since DeBord's strains are no longer in existence for comparison. Murray and Truant[17] suggest that *Mimeae* and *Bacterium anitratum* be included in the genus *Moraxella*. Whatever their ultimate classification, medical bacteriologists should be familiar with these organisms which occur frequently in a variety of clinical material.

The characteristics of *Bacterium anitratum* are now definitely established. Morphologically they appear as Gram negative diplococci, bacillary forms being rare in freshly isolated cultures. Colorless colonies on desoxycholate agar plates when fished to T.S.I. slants give an alkaline reaction, although in Durham tubes dextrose is fermented with acid in the outer tube only. No gas is produced. A single test can be used to confirm the identity of this organism. Although 1% lactose is not fermented, *Bacterium anitratum* does ferment 10% lactose agar slants. Investigation has failed to reveal an explanation for this unique reaction. However, it is a specific and useful test. The medium is easily prepared by adding 10% lactose and 1.5-2% agar-agar to an appropriate broth base, such as B.B.L. phenol red broth. Careful autoclaving at 10 pounds pressure is required to prevent break down of the highly concentrated carbohydrate during heat sterilization.

The differentiation of *Pseudomonas* species,[18] the identification of *Alcaligenes fecalis* without resorting to flagella stains, which are difficult and unreliable in inexperienced hands, and the identification of *Achromobacter*, all present difficulties, although the latter are rarely, if ever, of clinical significance. The difficulties are due to synonimy, incomplete knowledge and conflicting opinions, which

are well illustrated in the publications of Brisou[19] and Brisou, Morichau-Beauchant and Gimenez.[20] Further investigations must be undertaken before clarification of the taxonomy of these organisms is achieved.

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II. STREPTOCOCCI

Despite intensive studies by numerous investigators over many years, our knowledge of the streptococci is still incomplete, and definite problems exist in the differentiation of streptococci isolated from clinical material. The beta streptococci are well understood. They have been thoroughly studied both biochemically and serologically and are now classified serologically according to the Lancefield system on the basis of their "C" polysaccharide antigen. However, the grouping and typing of beta streptococci by the Lancefield method is not readily adaptable to the routine diagnostic laboratory, and sera for this procedure are available only to certain laboratories engaged in special investigations. Of the alpha and gamma streptococci and the anaerobic apecies much still remains to be learned. Incomplete knowledge and divergence of opinion has resulted in taxonomic confusion, and the medical bacteriologist, confronted with incomplete classification systems and varying interpretations cannot help but feel insecure in attempting to identify these organisms. Under these circumstances, what can be done today with streptococci isolated from clinical material that is practical and meaningful? First, procedures for the isolation of streptococci will be reviewed, and then differential methods that yield clinically significant results will be considered.

A. Isolation of Streptococci

Unfortunately, a number of misconceptions exist concerning the blood that should be used in preparing blood agar for the isolation of streptococci. In spite of well documented evidence to the contrary, [1,2,3,4,5] many bacteriologists feel that only rabbit blood is satisfactory, while others are firmly convinced that horse blood should be used. Human blood is frequently regarded as unsatisfactory. Actually, it makes little or no difference which blood is used, and the choice may well rest on availability rather than the superiority of one blood over another.

In choosing the blood to be used in preparing agar plates and in interpreting the results, the following facts should be taken into consideration:

1. Streptococci of all types can be isolated on suitable nutrient agar containing any one of a variety of bloods, those usually used being rabbit, horse, sheep and human.
2. Beta streptococci in both deep and surface colonies will produce clear-cut beta type hemolysis regardless of the kind of blood used, but the size of the zone of hemolysis will vary with the different types of blood.
3. Alpha and gamma streptococci vary in appearance according to the type of blood used in preparing the blood agar, a circumstance which is partially responsible for the confusion existing in the classification of these organisms. A particular streptococcus may be alpha on one type of blood agar and gamma on another; for example, *Streptococcus MG*. Variations also occur in the appearance of deep and surface colonies, for example, *Streptococcus fecalis* produces gamma surface colonies on rabbit and human blood agar while deep colonies in the same medium show green discoloration and slight partial hemolysis after 48-72 hours incubation. For this reason *Streptococcus fecalis* is described by some authors as a gamma streptococcus and by others as an alpha type.

The alpha appearance produced by streptococci also varies with the blood used. In general, on rabbit blood agar green discoloration is not a constant finding and many strains of alpha streptococci produce definite zones of hemolysis which grossly resemble those of beta streptococci, the fixed cells surrounding the alpha streptococcus colonies being detectable only by microscopic examination. On human blood agar the reverse is true, the hemolytic alpha types occurring less frequently and the majority of strains producing green discoloration that can be grossly recognized as alpha (viridans) in type. This difference is decidedly in favor of the use of human blood agar, since it allows the more rapid recognition of the majority of strains of alpha streptococci and simplifies their differentiation from beta types.

4. Inhibitory substance are present in some bloods against certain organisms but not against streptococci to any noticeable extent. Human blood has been suspected of inhibiting the growth of beta streptococci, due to antibodies which may be present against the various streptococceal antigens. Special studies and practical experience have shown that this is not the case and there is no inhibition of streptococci on human blood agar. (However, substances inhibiting the growth of *Hemophilus influenzae* are regularly present in sheep blood and occur to a varying extent in human blood. Fresh human blood agar gives poor and irregular growth of *Hemophilus influenzae*, but media prepared with old human blood, for example, outdated blood bank blood 2-6 weeks old, gives good growth of these fastidious Gram negative bacilli.[5])
5. The age of the blood used, within reasonable limits, is not a factor in preparing blood agar for the cultivation of streptococci, blood up to six weeks old having been found satisfactory.[5]

With these facts in mind, blood for the preparation of blood agar can be chosen according to availability and the facilities of the laboratory. For most hospital laboratories the blood bank is the most convenient source. Human blood bank blood, discarded because of a positive serologic test for syphilis, or outdated, is usually available in quantities far exceeding the demands of the laboratory. This solves the problem of obtaining suitable blood in a most practical manner.

Next arises the question of how blood agar plates are to be inoculated with the clinical material; that is, whether deep or surface inoculation is preferable. With one exception, surface streak plates are satisfactory and even more desirable, since they are quickly and easily prepared and have the added advantage that surface colonies are more readily differentiated than deep colonies. The exception to the exclusive use of streak plates for culturing clinical material is in the handling of throat cultures. *It has been definitely demonstrated that a streak plate cannot be depended upon to detect beta streptococci in throat cultures.* In a high percentage of cases no recognizable, isolated hemolytic colonies are detectable on streak plates, while deep pour plates show as many as 60-70 deep hemolytic colonies. Therefore, the following procedure is recommended for throat cultures:

1. Suspend the material from the throat swab in 4-6 ml. of sterile broth.
2. Streak a loopful of this suspension on a blood agar plate.
3. Prepare a blood agar pour plate as follows:
   a. Prepare a tube of fluid blood agar by adding 0.5-0.8 ml. of blood to approximately 12 ml. of a suitable agar base which

has been melted and cooled to 45-50 degrees C.

b. Add 1 loopful of the throat swab suspension to the fluid blood agar.

c. Mix well and pour into a sterile petri dish.

If this procedure is followed and both the streak and pour plates examined carefully after 24 and after 48 hours incubation, the percentage of beta streptococci isolated from throat cultures will be markedly increased. However, one word of caution should be added. If beta hemolytic, streptococcus-like colonies are found on blood agar plates, they should not be assumed to be those of beta streptococci without proper confirmation. A Gram stained smear should be made of suspicious colonies, particularity from throat cultures, in order to determine that the organism is definitely a streptococcus. This smear is necessary to eliminate the possibility that the colonies are those of other hemolytic organisms, the most common of which is *Hemophilus hemolyticus*. These pleomorphic Gram negative bacilli are frequently found in throat cultures, their colonies are identical with those of beta streptococci, and only a Gram stained smear can be relied on to distinguish the two organisms. After 48-72 hours incubation the hemolytic colonies of *Listerella*, a motile Gram positive bacillus, also resemble those of beta streptococci and a smear is required to differentiate them.

The next problem that arises in the isolation of streptococci is their separation from Gram negative bacilli. Species of *Enterobacteriaceae* and similar Gram negative bacilli frequently occur in clinical material together with Gram positive cocci and usually overgrow the cocci and make their isolation difficult, if not impossible, on ordinary blood agar. A number of substances have been used to inhibit the growth of these Gram negative bacilli, among them potassium tellurite, choral hydrate and sodium azide. These inhibitory agents have not been completely satisfactory, particularly due to their failure to inhibit *Proteus* and *Pseudomonas*.

The introduction of B.B.L. phenolethyl alcohol (P.E.A.) medium has offered a satisfactory solution to the problem. Blood agar prepared from this dehydrated medium will support good growth of streptococci, pneumococci and staphylococci while inhibiting the growth of most Gram negative bacilli completely. Some of the more resistant strains, particularly *Proteus* and *Pseudomonas* species, are not completely inhibited but grow poorly in minute colonies which are easily differentiated from the larger colonies of the Gram positive cocci.

In using P.E.A. agar certain precautions must be observed. Since the inhibitory agent, phenolethyl alcohol, is volatile, it is best to prepare the blood agar plates immediately after sterilization of the P.E.A. agar base, in order to avoid prolonged heating

or remelting of the medium. Plates should not be prepared too far in advance since long storage results in a decrease in concentration of the active ingredient and loss in selectivity of the medium. Care must also be taken in fishing colonies from P.E.A. agar. The Gram negative bacilli streaked on the surface of the plates with the original inoculum are inhibited but not killed. They may be visible as minute colonies or there may be no evidence of their presence. If colonies of Gram positive cocci are not fished very carefully and if the inoculating needle is swept over the surface of the agar, the resultant subculture may contain the original mixture of Gram negative bacilli and Gram positive cocci. However, if a straight inoculating needle and good technique are used, a pure culture of Gram positive cocci can be obtained with ease. It should also be noted that the hemolysis of streptococci on this medium is not always identical with that produced on ordinary blood agar, the hemolysis often being exaggerated on the phenolethyl alcohol medium.

A small laboratory may not feel justified in stocking a special medium for the separation of Gram positive cocci and Gram negative bacilli, in which case the ether method of eliminating Gram negative bacilli may prove useful. In this procedure, ½ ml. of ether is added to 1 ml. portion of the mixed culture. The mixture is well shaken and a loopful streaked immediately on a blood agar plate. A pure culture or well isolated colonies of the Gram positive coccus can thus be obtained without resorting to a special selective medium.

B. Differentiation of Streptococci

The initial step in the differentiation of streptococci is the determination of their effect on blood agar, that is, whether they are alpha (viridians), beta (hemolytic) or gamma (non-hemolytic) streptococci. Contrary to widespread opinion, this cannot be done in all cases by simple inspection of the blood agar plate. Gamma streptococci are easily recognized, producing no change in the blood around deep or surface colonies. The alpha streptococci which produce grossly detectable greening of the red blood cells are also immediately identifiable and present no problem. The difficulty arises in differentiating beta streptococci from those alpha streptococci which produce marked hemolysis of the red blood cells. With the naked eye these hemolytic alpha streptococci cannot be differentiated from the beta streptococci because the distinguishing factor, the presence of fixed, unhemolysed red blood cells immediately surrounding the colony, cannot be detected without the aid of the microscope. When these fixed cells are present in large numbers their green discoloration is grossly evident and indicates the alpha type of streptococcus, as noted above. When only a few fixed cells are present and there is considerable hemolysis, *only microscopic examination will differentiate these alpha strepto-*

cocci from beta streptococci, all of which produced clear zones of hemolysis with no red blood cells remaining around the colony.

The above discussion should point out the misrepresentation inherent in classifying streptococci by the descriptive terms "hemolytic," "viridans" and "non-hemolytic," rather than designating them accurately as "alpha," "beta" and "gamma." Hemolytic types include both alpha and beta strains and as a classification is too inclusive. Not all alpha streptococci produce the greening that would identify them as viridans, so that classification is too narrow. If streptococci are classified according to these descriptive terms, so called hemolytic strains will include hemolytic alpha streptococci which have important clinical differences from the beta streptococci with which they have been erroneously classified.

The essential microscopic examination of hemolytic colonies of streptococci can be carried out on both deep and surface colonies from streak or pour plates. For the study of deep colonies, the petri dish is inverted on the stage of the microscope, the low power objective used and colonies which can be brought into sharp focus selected for examination. For examination of surface colonies, the lid of the petri dish is removed and the low-power objective focused sharply on the edge of the colony. In both cases, clear, cell-free zones indicate beta type colonies, while the presence of unhemolysed red blood cells around and/or under the colony indicates alpha type colonies. With experience this differentiation, which should be carried out on all hemolytic streptococcus colonies, becomes a simple procedure. If the colonies are identified as beta in type, then the organism's identify as a beta streptococcus must be confirmed by Gram stained smear, as described in the preceeding section.

After a streptococcus has been correctly identified as alpha, beta or gamma in type, what further classification is important? As a practical routine procedure, the only other important step of clinical significance is the identification of enterococci, streptococci of intestinal origin which occur frequently in specimens from the peritoneum, pelvis and genito-urinary tract. Less frequently they are the cause of bacteremia and subacute bacterial endocarditis and are occasionally found in other cultures. Included in the enterococcus group are the beta streptococci of Lancefield Group D and *Streptococci fecalis*. On blood agar streak plates the latter produces gamma surface colonies, while in deep blood agar plates slight greening and weak alpha hemolysis is produced after 48-72 hours incubation. It is important to recognize these enterococci because of their well-known resistance to therapy. They are completely resistant to sulfonamides, penicillin and streptomycin, and are only slightly to moderately sensitive to other antibiotics: aureomycin, terramycin, tetracycline, chloro-

amphenicol and erythromycin. Because of these problems in the treatment of enterococcus infections, these resistant organisms should be differentiated from the other streptococci which are uniformly very sensitive to these therapeutic agents.

There are several tests which can be used to distinguish enterococci. Heat resistance, reduction of methylene blue milk and high salt tolerance as determined by growth in broth containing 6.5% sodium chloride are characteristics frequently used in identifying these organisms. As a substitute for, or as an addition to, these tests, the use of a comparatively new and efficient medium for the selection of enterococci is suggested. This is "S.F." *(Streptococcus fecalis)* broth obtainable in dehydrated form from the Baltimore Biological Laboratory. S.F. broth was developed some years ago for use by the sanitary bacteriologists in the testing of milk and water for fecal streptococci, and it is not widely known to medical bacteriologists. Its active ingredient is sodium azide which, in a concentration of 0.5%, inhibits the growth of Gram negative bacilli, staphylococci, pneumococci and streptococci with the exception of enterococci. The medium also contains as an indicator system dextrose and brom cresol purple. If the organism is an enterococcus and able to multiply in S.F. medium, the dextrose is fermented with production of acid and a resultant color change in the indicator after 24-48 hours incubation. Thus this medium offers an objective test not based solely on the presence or absence of growth, which is sometimes questionable. Experience has proven this S.F. medium to be reliable in the rapid identification of enterococci and its use can be recommended. It is especially valuable when used in conjunction with P.E.A. agar, since inoculations from P.E.A. plates to S.F. medium are assured of yielding growth of Gram positive cocci only, the Gram negative bacilli which may be picked up from the surface of the P.E.A. agar (see page 284) being inhibited in the S.F. broth.

In the study of alpha and gamma streptococci isolated from human clinical material, the identification of enterococci is the only clinically significant procedure that can be recommended at this time. As for the beta streptococci, it may be worthwhile to carry identification beyond the simple selection of enterococcus (serologic Group D) strains. This is particularly important in throat cultures, where it is often desirable to differentiate the pathogenic Lancefield Group A beta streptococci from those of other serologic groups which may represent only normal flora. As has been mentioned before (page 280), serologic grouping by the Lancefield method is neither practical nor possible for most clinical laboratories, so other means of differentiation must be used.

The serologic groups of beta streptococci most frequently encountered in human material are Groups A, B, C and D. The identification of Group D (enterococcus) strains has already been discussed. Group B beta streptococci can be identified by their hydrolysis of sodium hippurate, which is a simple laboratory procedure. The differentiation of Groups A and C by other than serologic means formerly presented an unsurmountable difficulty, since they are biochemically identical. Recently, however, Maxted[6] has presented a solution to this problem.

Maxted reports that the Lancefield Group A beta streptococci are more sensitive to bacitracin that are the other beta streptococci, and that this sensitivity can be used for the selection of Group A strains. He suggests the use of filter paper strips dipped in a 5 unit per ml. solution of bacitracin. These paper strips are then dehydrated and placed on the surface of inoculated blood agar plates as in the usual disc sensitivity test. A modified method, eliminating dehydration of the impregnated filter paper, has been found to work equally well. A sterile filter paper disc is dipped into a 1 unit per ml. solution of bacitracin and the wet disc placed immediately on the surface of the inoculated blood agar. Group A beta streptococci show a 15-17 mm. zone of inhibition, while the growth of streptococci of other serologic groups is not inhibited. The blood agar can be streaked with a pure broth culture of the beta streptococcus after isolation, or the plate can be inoculated directly from a colony and streaked so as to obtain uniform growth. This simple test for sensitivity to a low concentration of bacitracin has proven to be a practical and significant laboratory procedure and it is recommended for the identification of Lancefield Group A beta streptococci.

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III. NEISSERIA

There has been no greater improvement in diagnostic bacteriology than in the methods available for the cultivation of *Neisseria*. What was once a laborious and unreliable procedure has become simple and dependable. Good media, such as Difco "GC" chocolate agar prepared with dehydrated hemoglobin and Supplement A or B, render the *Neisseria* as easily cultivated as staphylococci. Methods for securing reduced oxygen tension have progressed from

the relatively involved CO2 jar, which required special apparatus, to the candle jar for which nothing more elaborate than an empty gallon mayonnaise jar is necessary. After easily growing *Neisseria* by this simple method, the oxydase test performed with a dilute solution of a dye such as para-aminodimethylaniline monohydrochloride will pick out the colonies of *Neisseria* from mixed cultures.

However simple the present procedure may be, there is one step in the isolation and identification of *Neisseria* where caution should be observed. It is inadvisable to accept an organism as a species of *Neisseria* on the basis of a smear from a colony showing Gram negative diplococci. The *Moraxella*, for example, can be confused morphologically with *Neisseria*. *Moraxella* is a relatively new genus, first proposed in 1939. A good description is to be found in the recent article of Murray and Truant.[1] The *Moraxella* are diplobacilli, morphologically resembling Gram negative diplococci and, like the *Neisseria*, are oxydase positive. They occur in throat and vaginal cultures and can be confused with *Neisseria meningitidis* and *Neisseria gonorrhoeae*.

Another Gram negative bacillus appearing regularly as a Gram-negative diplococcus is *Bacterium anitratum* (see preceding section on *Enterobacteriaceae*). *Bacterium anitratum* is oxydase negative, but in its freshly isolated "M" form occurs in marked diplococcus form indistinguishable from *Neisseria*. It occurs in the cervix and vagina and has been confused with *Neisseria gonorrhoeae*. In at least one instance, *Bacterium anitratum* isolated from the spinal fluid of a case of meningitis was originally mistaken for *Neisseria meningitidis*. In fact, a number of our original strains of *Bacterium anitratum* were identified as *Neisseria* on first isolation, an error that can easily occur.

Pasteurella multocida in its "M" form appears to be morphologically identical with *Neisseria*. This organism is usually regarded as an animal pathogen but human infections with it are being reported in increasing numbers, particularly from cases of bronchiectasis.[2] In material from the respiratory tract *Pasteurella multocida* may well be mistaken for species of *Neisseria* which are normally present in such specimens. Study of colony morphology, growth requirements and biochemical reactions will result in the proper identification of all of these diplococcus-like Gram negative bacilli, if medical bacteriologists are aware of their existence and the importance of differentiating them from *Neisseria*.

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IV. MYCOBACTERIUM TUBERCULOSIS

Methods for the isolation of *Mycobacterium tuberculosis* have reached a high level of efficiency. The accepted standard procedure of concentration with 4% sodium hydroxide yields good results. Media have been improved and excellent already prepared media are available commercially, for example, the Lowenstein-Jensen medium distributed by the Baltimore Biological Laboratory. However, the conventional media for the cultivation of tubercle bacilli, that is, the egg-base media such as Lowenstein-Jensen and A.T.S. (American Trudeau Society) media, are expensive to buy and are not simple to prepare. A new and radically different medium has been described within recent years by Tarshis[1] which promises to solve this problem. The formula for Tarshis medium is as follows:

Agar	1.5 gms.
Glycerol (analytical grade)	1.0 ml.
Human blood bank blood, outdated, with ACD solution	25.0 ml.
Distilled water	74.0 ml.
Penicillin	50 units per ml.

pH—approximately 6.8

The agar is disolved in the water and glycerol and the mixture sterilized in the autoclave, after which it is cooled to 45 degrees C in a water bath. The blood and penicillin are added, thoroughly mixed and the medium tubed in approximately 3.5 ml. amounts in sterile screw-cap tubes and allowed to harden into slants.

As can be seen, this medium is simplicity itself, yet Tarshis and his co-workers[2] and others have demonstrated that it is as satisfactory for cultivation of *Mycobacterium tuberculosis* from concentrated clinical specimens as any of the former media with elaborate formulae. Tarshis blood agar is inexpensive and easily prepared and can be stored for weeks in screw-cap tubes. It should prove very useful, especially in laboratories which desire to use a second medium along with one of the traditional egg-base media.

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V. BACTERIOLOGICAL STUDIES ON BLOOD BANK BLOOD

With the development of blood banking techniques and the widespread establishments of blood banks, a problem has arisen which concerns the bacteriology laboratory. There are numerous reports in the literature and many hospitals have had unfortunate experience with severe and some times fatal reactions following administration of bacterially contaminated bank blood. These

are, of course, of great concern to the blood banks, but there are also aspects of the problem which are of considerable interest to the bacteriology laboratory. It is the bacteriologist who has the responsibility for sterility tests on blood and for the identification of any contaminants which may be present. For this reason, the bacteriologist should be aware of the nature of the organisms which may be encountered and how the presence of these bacteria can be detected.

The best review of this problem is that of Pittman,[1] who reports bacteriological studies on eighteen such transfusion reactions, eleven of which were fatal and all of which were caused by blood contaminated with Gram negative bacilli. Pittman studied the organisms from thirteen of these cases, which included eight *Pseudomonas* species, two *Paracolobactrum aerogenoides* and three strains of *Escherichia freundii*. Other investigators have described reactions caused by *Micrococci, Bacilli, Streptococcus fecalis* and *diphtheroids*, but these Gram positive organisms are believed to be responsible for the milder febrile reactions. In severe and fatal cases Gram negative bacilli are usually implicated and of these, species of *Pseudomonas* are most common.

These *Pseudomonas* strains deserve special consideration, not only as the most frequent cause of transfusion reactions but also because of their unusual characteristics. Pittman describes them as producing yellow-green fluorescent pigment, utilizing citrate, proteolytic and with variable carbohydrate fermentations. They are not *Pseudomonas aeruginosa* because they fail to grow at high temperatures (37-41° C), do not produce blue pigment (pyocyanine) and differ in arrangement of flagella. She does not assign these organisms to any particular species since the taxonomy of the genus *Pseudomonas* is too confused at present to allow definite identification.

It should be noted that all the organisms studied by Pittman are able to utilize citrate as a sole source of carbon. This may account for their ability to grow in blood bank blood which is collected in citrate solution. It is interesting that citrate—negative organisms such as *Escherichia coli* were not found.

One of the most important facts brought out by Pittman's study and also stressed by other investigators is the temperature range for growth of these blood contaminants. Of the eight strains of *Pseudomonas*, two grew poorly and six failed to grow at 37° C, some were actually killed at incubator temperature, and all grew well from 2.5 to 30° C. The *Escherichia freundii* grew at 10-37° C and the paracolon strains from 2.5-37° C. In a fatal reaction not included in Pittman's series, a Gram negative bacillus, tentatively identified as an *Achromobacter* species, was isolated. This organism grew well at room temperature, poorly at refrigerator temperature and not at all in a 37° C incubator.

The ability of these organisms implicated in transfusion re-

actions to grow at the low temperature used for blood storage explains their presence in large numbers in stored blood bank blood. Most important for the bacteriology laboratory is the consideration of these temperature ranges in doing sterility tests on stored blood or blood which is suspected of having caused a transfusion reaction through bacterial contamination. Since the most common organisms in severe and fatal reactions grow poorly or not at all at incubator temperature, if sterility tests on blood are incubated in the conventional manner at 37° C, the presence of these cold-growing organisms will not be detected. Therefore, sterility tests on stored blood or plasma should be incubated at temperatures selected to permit the growth of any organism which may be present. Perhaps the simpliest procedure for the average laboratory is to inoculate three tubes of a suitable medium, for example thioglycollate medium, with 2-3 ml. samples of blood to be tested for sterility. Each of the three tubes should be incubated at a different temperature, one in the refrigerator at approximately 10° C, one at room temperature, and the third in the incubator at 37° C.

Another means of control of blood bank blood is the preparation of Gram stained smears of blood after storage. In fatal reactions following administration of contaminated blood, the blood has been found by smear to be teeming with Gram negative bacilli, innumerable organisms being present in each microscopic field. This is a regular finding in spite of the fact that the blood appears to be normal and there is no discoloration, hemolysis or other indication of the heavy bacterial growth that is present. Thus, microscopic examination of Gram stained blood smears will detect grossly contaminated blood and prevent severe and possible fatal transfusion reactions. However, failure to demonstrate the presence of bacteria in stained smears is not a guarantee of sterility. There is evidence that reactions can be produced with blood in which too few organisms are present to be demonstratable in stained preparations. Only sterility tests done in an adequate medium at appropriate temperatures can be relied upon to prove the sterility of stored blood bank blood.

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VI. CONCLUSION

In this review, which has been necessarily very limited in scope, a few new media and methods have been described. Emphasis has been placed on improvement in procedures and the problems arising from these improvements. Such suggestions for modifications in methods raises the question of when, why and how medical bacteriologists should initiate changes in their own laboratory procedures. Confronted with seemingly endless new

media, suggestions and recommendations, how can the technologists choose methods best adapted to the facilities and the demands placed upon their particular laboratories.

Unfortunately this problem is often approached in one of two ways. Many laboratories try practically everything that appears in print, quite uncritically, with the result that their procedures are over-complicated and cluttered with unnecessary details. Other laboratories make no changes whatsoever and cling to out-moded methods when better ones are available. The advice offered by Alexander Pope is still good and very appropriate:

> "Be not the first by whom the new are tried
> Nor yet the last to lay the old aside"
>
> Essay on Criticism, Part II.

Procedures should be well tried and proven before their acceptance in a clinical laboratory. If possible, it is desirable to run a duplicate series before introducing a new technique, comparing the old with the new method under the conditions in which it will be put to actual use. It is helpful to have available a collection of typical stock cultures with which such studies can be done. A set of carefully selected cultures is easy to maintain and invaluable in testing new media, methods and antibiotics.

Sometimes checking a new or modified procedure involves considerable time and trouble, but the results frequently justify the effort. For example, a few years ago it seemed desirable to compare methods for the handling of urine cultures, an important procedure considering the large number received in a clinical laboratory. The conventional method involves centrifuging the urine specimen and inoculating the sediment on appropriate plates and broth. Centrifugation of a large number of specimens is a time consuming process and it would be a decided advantage if it could be eliminated. An increasing number of laboratories were omitting this step and using the whole urine for the inoculation of the media. But if centrifugation were omitted, would clinically significant organisms be missed? This question seemed important enough to warrant a study to compare the two methods. Therefore, duplicate sets of cultures were made on 150 urine specimens, one set being inoculated with the whole urine and the other set with the centrifuged sediment. Marschall (unpublished data) made a comparison of the results and found that 94% of the series yielded identical results; either both sets of cultures were negative or the same organism was isolated from each. In 6% organisms were isolated from the centrifuged specimen and not from the whole urine. The great majority of these organisms were *Staphylococcus albus;* the others were either frank contaminants or of doubtful significance. On the basis of these results, centrifugation of urine specimens was

stopped. Several years of experience has now confirmed these findings and justified the change in procedure. The percentage of contaminants has markedly decreased and to our knowledge in only one of over ten thousand urine specimens has a significant organism been missed by culturing the whole urine.

If it is impossible for a laboratory to check new methods, then it is imperative that the bacteriologist maintain close contact with fellow workers in other institutions, particularly in the large teaching centers where research is more easily undertaken. It is unwise to maintain an isolated laboratory existence. Good "shop talk" with colleagues is invaluable in keeping well informed on recent developments. Therefore, membership and active participation in scientific societies is important, and the meetings of such societies should be planned so that the members have ample opportunity for informal exchange of information and experience.

NOMINATIONS COMMITTEE

The following officers of the American Society of Medical Technologists will complete their term of office in June, 1956, at which time the House of Delegates will elect replacements:

President-elect.....C. Patton Steele
Recording Secretary*.....Ellen Anderson
Board of Directors.....Leanor Haley - Rose Hackman
Board of Approved Schools..Sister Mary Clare (Heath) of New York

Names of qualified candidates for these offices and positions may be submitted by constituent societies or from any active member of the Society. The candidate must be an active member in good standing and must consent to serve if elected. Send names of proposed candidates to the Secretary of the Nominations Committee: Mrs. Katherine Muir, 1816 Rosedale, Louisville, Kentucky. The proper forms will then be sent to the candidate for completion and copies of these forms will be circulated to the members of the nominations committee in order that they may prepare a slate of at least two names for each office or other position to be filled by election of the House of Delegates. The completed forms submitted by the candidate must be in the hands of the Secretary of the Nominations Committee by December 1, 1955.

The Nominations Committee is desirous of obtaining the names of as many candidates as possible in order to select a desirable slate. Please send us the names of members who have the qualifications and leadership ability that is needed by national officers. The names and biographical information concerning the candidates selected by the Nominations Committee will appear in the April NEWS RELEASE.

* May be re-elected.

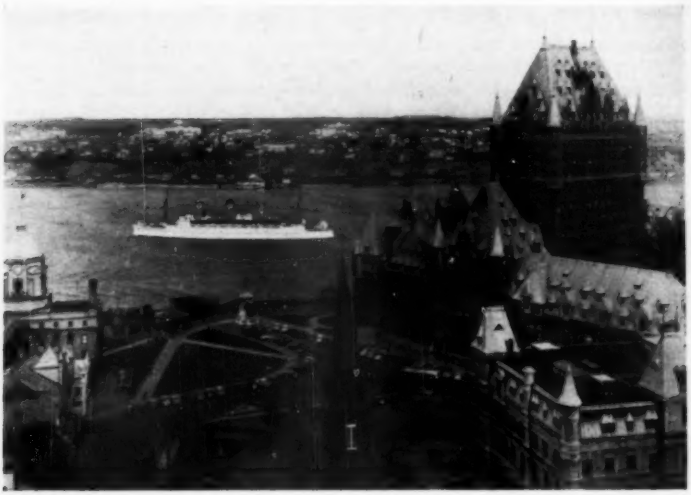
GENEVIEVE STOUT, M.T. (ASCP)
Chairman, Nominations Committee

THE GAVEL

Plans are progressing at a rapid pace toward a history making event in the First North American Conference of Medical Laboratory Technologists which will be held June 17-22, 1956, in Quebec City, Canada. NOW is the time to start working on your paper to enter in the competition for the numerous awards made available through our friends and our own society. (Winners could enjoy more foreign travel.) This is also the time to acquaint your hospital administrator or your "boss" with the advantages to them by their making it possible for you to attend this First North American Conference of Medical Laboratory Technologists. (As most of you realize the forward looking hospitals, and the up-to-date "bosses" are reaping dividends by making it possible for their medical technologists to take advantage of our meetings.) Not only do you have the opportunity to hear about new procedures and techniques, examine the latest in equipment, but also to talk with those who can help you solve your problems in the clinical laboratory. Another thought regarding this meeting should be mentioned, and that is to send your choice for the new slate of officers and members of the Board of Directors to the Nominations Committee in plenty of time to allow the committee to prepare a slate that will further the aims of your professional society. SERVICE THROUGH COOPERATION has been adopted as the theme of this First North American Conference of Medical Laboratory Technologists.

Barbara Isbell, M.T. (ASCP), President

(See page 253 for Program Committee Announcement)



Chateau Frontenac, Quebec, P. Q., Canada—The St. Lawrence River in the background. Headquarters—1st North American Conference of Medical Laboratory Technologists, June 17-22, 1956.

ABSTRACTS

PROPOSAL FOR THE DISTRIBUTION OF A CERTIFIED STANDARD FOR USE IN HEMOGLOBINOMETRY

R. Keith Cannon. (Division of Medical Sciences, National Academy of Sciences—National Research Council) J. Lab. Clin. Med., 46, 135-40 (1955).

This report proposes a plan for distribution of a certified hemoglobin standard and advocates the wide adoption of a recommended analytical procedure. The recommendations included:

1. A Standard of Reference in the form of a preparation of crystalline human hemoglobin prepared by Drabkin's method. The solution shall contain 1 milliatom of hemoglobin iron per liter and shall have a millimolar extinction coefficient of 11.5 at a wave length of 540 mu when measured as cyanmethemoglobin. Certification of this standard shall be made by National Institutes of Health, Army Medical Service Graduate School, National Bureau of Standards, and Dr. Drabkin's laboratory at the University of Pennsylvania.
2. The iron content of hemoglobin shall be 0.335 per cent, corresponding with an equivalent weight for hemoglobin of 16,700 per atom of iron and with an oxygen capacity of 1.34 ml. per gram of hemoglobin.
3. A certified solution of cyanmethemoglobin shall be a Standard for Distribution and shall be packaged in three separate solutions containing concentrations of approximately 20, 40 and 60 mg. of hemoglobin in the form of cyanmethemoglobin per 100 ml (corresponding to 1-250 dilutions of blood containing approximately 5, 10 and 15 gm. of hemoglobin per 100 ml.).
4. Drabkin's method (cyanmethemoglobin) is recommended for adoption by clinical laboratories. This method employs a single solution containing potassium ferricyanide and potassium cyanide, which converts the hemoglobin in blood quantitatively to cyanmethemoglobin. The ferricyanide converts the hemoglobin iron from the ferrous to the ferric state to form methemoglobin, which then combines with potassium cyanide to produce the stable pigment cyanmethemoglobin (the concentration of cyanide in the reagent is 52 mgs. of potassium cyanide per liter—its lethal dose for man approaches four liters).

A field trial of one year was agreed on by the panel.

A SIMPLIFIED DYE METHOD FOR ESTIMATING PLASMA VOLUME

Hugh A. Frank and Marie H. Carr. (Veterans Administration Hospital and University of Kansas School of Medicine) J. Lab. Clin. Med., 45, 977-80 (1955).

Plasma volume with Evans blue dye (T-1824) using some of the principles of radioactive tracer techniques is described. Aliquots of diluted dye are determined. All samples are prepared with the patient's own serum which eliminates the problems due to differing optical densities of T-1824 in different solvents.

THE INTERACTION OF SERUM WITH ERYTHROCYTES MODIFIED BY NEWCASTLE DISEASE VIRUS

Alfred S. Evans. (University of Wisconsin) J. Immunol., 74, 391-6 (1955).

The hemagglutinin and the cell-sensitizing substance of the virus are shown not to be identical and that the latter probably represents more than one component. One red cell sensitization which may occur results in agglutination by immune serum, the other agglutination by infectious mononucleosis serum. A useful genetic marker may be found in the variation of the sensitizing component for infectious mononucleosis serum and that of the NDV strain which persists through passage at limiting dilutions.

BLOOD GROUP FACTORS IN THE RABBIT

Carl Cohen. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor) J. Immunol., 74, 432-8 (1955).

The rabbits used in the preparation of immune testing sera were of various races and strains. The ages of the donors at the start of isoimmunization ranged from early maturity to one year. Weight and sex of the animals was not regarded. The donor blood was obtained aseptically by cardiac puncture and introduced into a sterile vaccine vial containing 3% sodium citrate solution and when kept sterile it was suitable for injection for as long as two weeks. Two other methods were used later, a B and D vacutainer device with a 24 or 25 gauge needle was found more satisfactory. By this method 30 ml. or more of blood could be obtained from the median artery of the ear aseptically with less trouble and risk to the animal whether the donor or recipient. Hemagglutination, absorption and elution tests were carried out. Typing sera were prepared for at least six specific blood group substances present in the rabbit. It was shown that the animal may have none to all six of the factors; some had A only; some, C only; some, E only; some, F only; some, H only; and some none of the known agglutinogens. It was proposed that for genetic interest this laboratory be considered the rabbit blood-group reference laboratory and that its stock be used for comparison with blood-group factors isolated in other laboratories.

INCIDENCE OF ANTIBODIES TO SALMONELLAE AMONG THE POPULATION OF THE BRONX, N. Y.

A. J. Weil and Ivan Saphra. (Bronx Hospital, Beth Israel Hospital and the Joseph and Helen Yeamans Levy Foundation) J. Immunol., 74, 485-7 (1955).

Among the tests carried out the antigens used covered the great majority of salmonellae presently met in the United States. It was pointed out that salmonellae and Flexner bacilli share minor antigenic factors. A low incidence of agglutinating antibodies was found with these Salmonella antigens; groups B, C_1, C_2, and D, and flagellar b, d, i, and 1, 5.

ANTIBODIES TO SHIGELLAE IN NORMAL HUMAN SERA

A. J. Weil and J. Felsen. (Bronx Hospital) J. Immunol., 74, 488-93 (1955).

It was noted that the consensus of the workers is that agglutinating serum antibodies against shigellae found in patients with negative histories is frequent but that the reactivity is essentially restricted to the mannitol-fermenting group. 300 to 369 specimens were tested from patients in a population where the incidence of shigellosis is low. Agglutinating antibodies versus types 1, 2, 3 and 4, of Sh. flexneri were found with frequency. Rare antibodies were found versus Sh. flexneri type 6, Sh. sonnei, Sh. dysenteriae type 2 (ambigua) and the alkalescens bacillus. Normal Flexner antibodies were against the group antigen rather than type-characterizing antigens. Cold agglutinins and non-agglutinating (univalent) antibodies were also found.

USE OF ISOTOPE-LABELLED RED CELLS TO DEMONSTRATE INCOMPATIBILITY IN VIVO

P. L. Morrison and Marie Cutbush. (Postgraduate Medical School of London) Lancet I, 1290-5 (1955).

A biologic test to determine the compatibility of blood in vivo is given. About 1 ml. quantities of red cells were labelled with either ^{32}P or with ^{51}Cr and injected. The survival of the transfused cells was then followed. ^{32}P was suitable when the survival was to be followed for an hour or so; ^{51}Cr must be used when the survival is to be followed for days or weeks. Incompatibility was shown by two main patterns:

1. (a) 50-100% of cells destroyed in 10 minutes
   (b) surviving cells much more slowly destroyed
   (c) this type of elimination occurred when the recipitnes serum contained anti-Fy^a, anti-Le^a, or anti-P.
2. (a) cells eliminated at a steady rate, half-period of 20-30 minutes
   (b) this type of elimination was found in recipients whose serum contained anti-Rh or anti-Kell.

BORIC ACID POISONING TREATED BY EXCHANGE TRANSFUSIONS

Thomas R. Boggs and Harry G. Anrode. (Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine) Pediatrics, 16, 109-14 (1955).

Exchange transfusions were successfully used for an infant accidentally poisoned with boric acid. A qualitative test using turmeric paper first revealed the presence of boric acid in the blood and urine. A semi-quantitative technique and a quantitative technique for the determination of boric acid are given. The method of Hatcher and Wilcox for the determination of boric acid in plant materials was modified for use with serum, packed cells and urine.

OBSERVATION ON THE CONVALESCENT PHASE OF ERYTHROBLASTOSIS FETALIS

Carol B. Hyman and Phillip Sturgeon. (Los Angeles Children's Hospital and University of Southern California School of Medicine) Pediatrics, 16, 15-23 (1955).

The natural course of convalescence in erythroblastosis fetalis and variations resulting from therapy are discussed. Twenty infants demonstrating D (Rh_0) incompatible erythroblastosis fetalis were treated and studied. An increase in total body hemoglobin rather than blood hemoglobin concentration is an earlier criterion of the status of the regeneration of the infant's blood. After the acute hemolytic period has passed, the slow continuing fall and plateau of the hemoglobin curve is a result of the infant's rapidly increasing blood volume rather than abnormal blood destruction. Many unnecessary blood transfusions may be avoided during this convalescent period by noting these statements. That there is an initial aregenerative period in erythropoiesis during convalescence is demonstrated by the delay in the appearance of reticulocytosis and in the return of the Rh test to positive.

LABORATORY DIAGNOSIS OF THE PLATYHELMINTH INFECTIONS

Marion Hood, Ph. D. Microbiologist, Department of Pathology, Charity Hospital, New Orleans, Louisiana.

Members of the Platyhelminthes are only occasionally found in the United States. This increases the responsibility of the technologist to keep these parasites in mind. The morphology and life cycles of the various Platyhelminthes must be understood and the most satisfactory diagnostic procedures known, so these infections can be recognized when encountered.

BY LAWS

AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

Revised 1953
Amended 1954-1955

BY-LAWS
OF THE
AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

ARTICLE I
Name

The name of this corporation is American Society of Medical Technologists.

ARTICLE II
Purposes

The purposes of this Society are to promote higher standards in clinical laboratory methods and research; to elevate the status of those specializing in medical laboratory technique; to create mutual understanding between the medical technologist and physicians and all others who are employed in the interest of individual or public health; to issue charters to constituent societies; to promote the mutual aid and benefits of its members; in general to do anything and everything necessary and proper to the conduct of a society of this nature, and for the purpose of attaining or furthering any of its objects to do any and all other acts and things, and to exercise any and all other powers which now or hereafter may be authorized by law.

ARTICLE III
Constituent and Branch Societies

Section 1. This Society shall be composed of constituent societies and of such branch societies as shall be organized and sponsored by the constituent societies.

Section 2. There may be one, and only one, constituent society in each geographically defined state, District of Columbia, territory, possession or dependency of the United States and in each foreign nation. Any number of branch societies may be organized by a constituent society within the boundary of its geographical area.

Section 3. The name of each constituent society shall contain the geographical location thereof and the words medical technologists, or the equivalent of these words. A constituent society may use the name of division of the American Society of Medical Technologists.

Section 4. A constituent society can be organized by three or more persons eligible for active membership in this Society. These persons shall petition for permission to organize a constituent society on a form supplied by the executive secretary which shall be submitted to the chairman of the Board of Directors and be approved by that Board. The petition shall be accompanied by an application fee of $5.00. When the petition has been approved, six copies of their proposed constitution and by-laws or, if the group wishes to become incorporated, six copies of their proposed articles of incorporation and by-laws shall be submitted to the constitution and by-laws committee of this Society accompanied by a list of officers and charter members. The committee shall bring a report and a recommendation concerning the chartering of this group to the House of Delegates at the next Annual Session of the Society.

Section 5. A charter for the organization of a constituent society may be granted only by the House of Delegates.

Section 6. The duties and functions of a constituent society are defined as follows:

(a) It shall send to the executive secretary of this Society the names and addresses of its officers not more than two weeks after their election, and the names and addresses of its committee chairmen not more than two weeks after their appointment.

(b) It shall send dues to the executive secretary within two weeks after members have paid, together with a duplicate, typewritten, classified list of such members with their addresses and registry numbers or degree statuses.

(c) It shall provide for the types and qualifications for membership except that it may grant active membership only to persons who meet the requirements for active membership in this Society, that only active members shall have the privilege of voting and holding office in the Society; and all members of a constituent society shall abide by the code of ethics of this Society.

(d) It shall sustain its status with this Society by maintaining a constitution and by-laws in harmony with the By-Laws of this Society and by maintaining adequate supervision over the branch societies which have been organized under its direction to insure that their constitutions and by-laws are kept in harmony with the By-Laws of this Society. The types of membership and qualifications for membership in a branch society shall be determined by the constituent society under which it is organized, except that all members of a branch society must abide by the Code of Ethics of this Society.

(e) It shall submit to the chairman of the constitution and by-laws committee of this Society twelve copies of all proposed amendments to the constitution and by-laws of the constituent society at least sixty days before the approval of such committee is desired. These amendments should be submitted in context. When the proposed amendments have been approved they may be adopted. A constituent society shall report the adoption of any amendments to its constitution and by-laws to the chairman of the constitution and by-laws committee and must submit a copy of its amended constitution and by-laws for filing in the executive office of this Society within two weeks after their adoption.

(f) It shall send annually before September 15th to the executive secretary a charter fee of $1.00 which provides for the renewal of its charter.

Section 7. This Society shall be empowered to revoke the charter of a constituent society by a two-thirds majority vote of the House of Delegates provided the Board of Directors by a two-thirds vote favors the revocation of such charter and provided, further, that such constituent society shall have had at least thirty days notice of the charges filed against it and shall have had an opportunity to be heard and to defend itself before the House of Delegates. The charter of a constituent society may be revoked for any of the following causes:

(a) Failure of such society to require that all its members abide by the Code of Ethics of this Society.

(b) Adoption by the constituent society of policies or procedures contrary to the purposes of this Society.

(c) Failure of the constituent society to cooperate reasonably with this Society.

Section 8. Any constituent society whose charter has been revoked may be reinstated by a two-thirds vote of the House of Delegates upon application, provided such society is no longer in default and pays all expenses incurred, plus a reinstatement fee of five dollars ($5.00).

Section 9. Any constituent society shall revoke the charter of a branch society organized under its direction for failure of the branch society to comply with both the Code of Ethics of this Society and the By-Laws under which the branch society was organized and amendments thereto.

ARTICLE IV
Membership

Section 1. The membership of this Society shall consist of the following classes:

(a) Active Member. Any person who (1) is registered M.T. (ASCP) by the Registry of Medical Technologists of the American Society of Clinical Pathologists and is in good standing with that Registry, or (2) possesses a master's or higher degree in microbiology, biochemistry, hematology, histology, parasitology, or serology from an accredited college, or (3) holds a certificate in chemistry or microbiology from the Registry of Medical Technologists of the American Society of Clinical Pathologists, and is in good standing with that Registry, or (4) holds a limited certificate in histologic technique from the above mentioned Registry and has ninety or more semester credit hours from an accredited college of which at least twenty-seven hours are in science including chemistry and/or biology, with lecture and laboratory in each, shall be eligible for active membership in this Society provided, further, that he is an active member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An active member shall have all the rights and privileges of this Society.

(b) Affiliate Member. Any person who holds a certificate from and is in good standing with the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists without meeting the requirements for active membership in this Society shall be eligible for affiliate membership in this Society provided that he is a member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An affiliate member is entitled to all the privileges of membership in this Society with the exception of voting, holding office, and serving on any Board or standing committee of this Society.

(c) Student Member. Any person enrolled in an approved School of Medical Technology is eligible for Student Membership in this Society. He may enjoy this membership until he becomes a registered M.T. (ASCP) by the Board of Registry of Medical Technologists of ASCP or until he becomes ineligible to take the examination for such registration. Upon successful completion of the Registry examination he shall automatically be billed for Active Membership in this Society. A student member is entitled to all the privileges of membership in this Society except voting, holding office, and serving on any Board or standing committee of this Society.

(d) Honorary Member. Any person who has made an outstanding contribution to the field of laboratory medicine by his research or by his service to the profession of medical technology may be recommended for honorary membership in this Society. Recommendation for honorary membership may be made by a constituent society or by the Board of Directors. A recommendation accompanied by qualifications shall be sent to the recording secretary of this Society not less than thirty days before the Annual Session. Honorary members shall be elected by a two-thirds vote of the House of Delegates. They shall be elected every third year beginning in 1954. The year when honorary members are elected each constituent society and also the Board of Directors shall be entitled to present the name of only one candidate for this honor. Honorary members shall not exceed in number one per cent of the active members of the Society in good standing at the time of their election to honorary membership. An honorary member shall be informed of his election by the recording secretary who shall send him a certificate of honorary membership signed by the president and the recording secretary. He shall enjoy all the privileges of membership except voting, holding office and serving on any Board or standing committee of this Society. He shall be exempt from payment of annual dues.

Section 2. Application for active or affiliate membership in this Society shall be made on a form prescribed by this Society. The transmittal to the executive secretary of a list of active and affiliate members in good standing in a constituent society, together with their annual dues shall establish them as members of this Society.

Section 3. Application for student membership in this Society shall be made on a form prescribed by this Society. The transmittal of this application to the executive secretary of this Society together with the required amount of dues shall establish him as a student member of this Society.

Section 4. Any member of this Society whose conduct is detrimental to this Society may be expelled by a two-thirds vote of the House of Delegates provided he has been given thirty days notice, signed by a majority of the members of the Board of Directors and sent by registered mail, stating the charges filed against him and informing him of the date of the meeting when his expulsion will be voted upon. The member so charged shall have the privilege of appearing before the House of Delegates and he may present his defense in person or through other active members of the Society of his selection.

Section 5. Any person who has been expelled from membership may be reinstated upon adoption by a two-thirds vote of the House of Delegates of his application for membership, provided the same is accompanied by annual dues to this Society plus a reinstatement fee of five dollars ($5.00).

ARTICLE V

Code of Ethics

Section 1. A member of this Society shall at all times work only under the direction and supervision of a pathologist or duly qualified doctor of medicine or specialist in one of the divisions of clinical pathology, such qualifications being determined on the basis of accepted medical ethics.

Section 2. A member of this Society shall make no diagnosis or interpretations other than those in the reports prepared by him.

Section 3. A member of this Society shall not advise physicians or others how to treat disease.

Section 4. A member of this Society shall not train students without supervision of a clinical pathologist.

Section 5. A member of this Society shall not engage in laboratory work independent of qualified supervision (as provided in Section 1) nor shall he operate an independent laboratory.

Section 6. It is ethical to perform laboratory work on a commission basis under contract with a public health, research, or clinical laboratory when such work is done as provided in Section 1 above and when all contractual agreements are approved and signed by the director of the organization contracting for such services.

ARTICLE VI

Meetings

Section 1. The Society shall hold an Annual Session which shall include one or more general or scientific meetings of the members and not less than two meetings of the House of Delegates at least two of which shall be held on different days. The Annual Session shall be held at the time and place determined by the House of Delegates at any preceding Annual Session. Emergency changes concerning the time and place of an Annual Session may be made by the Board of Directors.

Section 2. The Board of Directors shall meet within the week following the sine die adjournment of the House of Delegates to transact necessary business and shall hold a meeting within the two weeks prior to the first meeting of the House of Delegates at each Annual Session.

Section 3. The Advisory Council shall meet within the week following the sine die adjournment of the House of Delegates to transact necessary business and shall hold a meeting within two weeks prior to the first meeting of the House of Delegates of each Annual Session.

ARTICLE VII
Finances

Section 1. The annual dues for active and affiliate membership in this Society shall be eight dollars ($8.00) per annum.

Section 2. The dues for student members shall be on the basis of twenty-five cents ($.25) a month, until such time as he shall be eligible for Active Membership in this Society. Dues are to be paid as specified on the application form. The full amount due at that time shall accompany the application for membership, and shall be sent to the Executive Secretary of this Society. He may renew his student membership so long as he is eligible for registration.

Section 3. Dues to this Society shall be payable to the treasurer of the constituent society of the geographical area in which the member is employed or resides, or, in the event no such constituent society exists, to the executive secretary of this Society. Dues are payable on or before July 1 of each fiscal year.

Section 4. (a) Any active or affiliate member who cannot pay dues for the current fiscal year shall notify this Society by sending written notification direct to the executive secretary not later than September 1st of that year.

(b) Any active or affiliate member who fails to pay annual dues to this Society by September 1st shall have his name removed from the membership list.

(c) A person whose name has been removed from the membership list of this Society may be reinstated at any time provided he meets the requirements for membership of both his constituent society and this Society. Unless a person has previously resigned, to be reinstated in this Society he shall pay current annual dues to his constituent society with any back dues or reinstatement fee the constituent society may require, and current year's dues to this Society plus a reinstatement fee of two dollars to this Society.

(d) Any active or affiliate member who changes his residence or place of employment to another state, territory or nation shall become a member of the constituent society of his new residence or place of employment for the remainder of the fiscal year without further payment of dues.

Section 5. A new applicant elected to active or affiliate membership in this Society after January 1st and prior to May 1st shall pay one-half of the annual dues to this Society for the remainder of the fiscal year.

Section 6. An applicant elected to active or affiliate membership in this Society between May 1st and June 30th shall pay annual dues for one year which shall entitle him to all the privileges of his respective membership for a period up to fourteen months. He shall pay no further dues to this Society until the second fiscal year following the date of his application.

ARTICLE VIII
Officers

Section 1. The officers of this Society shall be president, president-elect, recording secretary and treasurer.

(a) The president shall be the chief executive of this Society and shall preside at all meetings of the Board of Directors and of the House of Delegates. He shall be an exofficio member of all standing and special committees except the Nominations Committee. He shall set up such standing committees as are provided for in the By-Laws except the Nominations Committee. He shall appoint with the approval of the Board of Directors, such special committees as are needed.

(b) The president-elect shall, during his term of office, familiarize himself with the duties which shall devolve upon him as president. In the event of the death, resignation or total disability of the president, the president-elect shall perform the duties of, and have the same authority as, the president.

(c) (1) The recording secretary shall act as secretary at all meetings of the Board of Directors and of the House of Delegates.

(2) He shall keep in permanent form in his possession a record of all minutes taken at all meetings at which he serves, and shall provide each of the members of the Board of Directors and the executive secretary with a copy of these minutes within thirty days after the close of the Annual Session at which the minutes were taken.

(3) He shall, assisted by the Minutes Committee prepare an abstract of the minutes to be published and distributed to the membership.

(4) He shall carry on such correspondence as shall be authorized by the House of Delegates and Board of Directors of this Society.

(5) To aid the recording secretary in the keeping of an accurate record of the annual meetings of the House of Delegates the Society shall employ a mechanical recording device or an expert stenographer to record all the proceedings of the meetings of the House of Delegates. Such record shall be delivered to the president of this Society by the stenographer within sixty days after the close of an Annual Session. The president shall within sixty days after receipt of the record transmit the same to the executive secretary to be filed in the executive office.

(6) In the event of the simultaneous inability of both the president and the president-elect to perform the duties of president, the recording secretary shall perform the duties of, and have the same authority as, the president.

(d) The treasurer shall receive, record and have charge of all funds of this Society. He shall deposit these funds in a bank designated by the Board of Directors and pay all bills approved by the Board of Directors or the House of Delegates upon receipt of vouchers signed by the chairman of the Board. He shall be bonded at the expense of the Society. He shall, within sixty days previous to the Annual Session, have his accounts audited by a public accountant at the expense of the Society. He shall submit to the House of Delegates at each Annual Session an itemized statement of all receipts and expenditures of the Society for the year just ended, and a copy of this itemized statement shall be distributed to each delegate prior to the reading of the report. At the end of his term of office he shall have a final interim audit of his accounts and shall deliver his current records and all cash on hand to his successor. All other records in his possession shall be transmitted to the executive secretary to be filed in the executive office.

(e) At the end of the term of office by expiration or otherwise each officer, except the treasurer, each Board member and Council member, shall deliver his official records and correspondence to his successor for transmittal to the executive secretary to be filed in the executive office.

Section 2. Eligibility for Office. Any person who is, and for at least three years prior to his election or appointment has been, an active member of this Society shall be eligible to hold office.

Section 3. Method of Election.

(a) All officers of this Society shall be elected by the House of Delegates at the Annual Session of this Society.

(b) An official list of nominees for each office shall be presented to the House of Delegates by the Nominations Committee, the list of candidates and their qualifications having been sent to each member of this Society at least sixty days before the Annual Session. Nominations may be made from the floor. The qualifications of all persons nominated from the floor shall be presented to the House of Delegates at the time such nominations are made.

(c) The election of officers by the House of Delegates shall be conducted

by an Elections Committee consisting of five members of the House of Delegates appointed by the chair.

(d) Election shall be by ballot and a majority of votes cast elects. If none of the candidates for a certain office receives a majority vote on the first ballot the candidate with the lowest number shall be eliminated and balloting shall continue until a candidate receives a majority of the votes cast. In the event of a tie, election shall be determined by lot.

Section 4. Term of Office.

(a) The president-elect shall be elected for a term of one year and, except in the event of a vacancy in the office of president by death, resignation or total disability, he shall serve for one year as president-elect and then automatically succeed to the office of president.

(b) The recording secretary shall be elected for a term of one year. He may be reelected. He shall not serve in this office for more than two years.

(c) The treasurer shall be elected for a term of three years. He may be reelected. He shall not serve in this office for more than six years.

(d) The term of office of any officer of this Society elected at an Annual Session shall begin with the sine die adjournment of the House of Delegates at such Annual Session.

Section 5. Filling of Vacancies.

(a) In the event of the death, resignation or total disability of the president, the president-elect shall become the president of this Society for the remainder of the year of the president's term of office and for the following year, and the Board of Directors shall elect by majority vote a president-elect to serve for the remainder of the year in which the vacancy occurs. At the next Annual Session following the occurrence of the vacancy in the office of president, the House of Delegates shall elect a president-elect for a term of one year.

(b) In the event of the simultaneous removal during their term of office of both the president and the president-elect by death, resignation or total disability the recording secretary shall become the president of this Society for the remainder of their term of office. The Board of Directors shall by majority vote elect a president-elect from the slate of nominees for that office from the previous election and a recording secretary from the slate of nominees for that office from the previous election, both of whom shall be elected to serve for the remainder of the year in which the vacancies occur.

(c) In the event of removal from office of the recording secretary by death, resignation or total disability the Board of Directors shall by majority vote elect a recording secretary from the slate of nominees for that office from the previous election to serve for the remainder of the year in which the vacancy occurs.

(d) In the event of removal from office of the treasurer by death, resignation or total disability the Board of Directors shall by majority vote elect a treasurer from the slate of nominees for that office from the previous election to serve for the remainder of the term of office for which his predecessor was elected.

ARTICLE IX
Executive Secretary

Section 1. The executive secretary shall be elected by the Board of Directors, shall serve under a written contract, and, subject to the action of the House of Delegates, shall be under the supervision and control of the Board. He shall, under the direction and control of the House of Delegates and the Board of Directors, maintain a central office for the Society in which the records, properties, bonds and legal papers of the Society shall be preserved in properly maintained files. He shall do the administrative work necessary to the operation of the Society and shall be the business manager of the official publication.

Section 2. The executive secretary shall contract for technical exhibits for the Annual Session of the Society and, working with the local chairman of exhibits, shall arrange for all technical exhibits. All receipts from technical exhibits shall go into the treasury of this Society.

Section 3. The Executive Secretary shall be included as a non-voting member of the Board of Directors and of the Advisory Council.

Section 4. The Executive Secretary shall transmit to the president, the president-elect and the recording secretary of this Society the names of the officers and committee chairmen of Constituent Societies within one week after their receipt.

ARTICLE X
Board of Directors

Section 1. The Board of Directors shall consist of the president, the president-elect, the recording secretary, the treasurer, the immediate past-president and six members of this Society elected by the House of Delegates.

Section 2. The Board of Directors shall represent the Society when the House of Delegates is not in session and only when urgent business necessitates immediate action that cannot wait until the House of Delegates reconvenes. In no case, except in an emergency, shall the Board of Directors transact business that vitally affects the interests of this Society and in no case shall the Board of Directors take any action which binds the Society beyond the next ensuing Annual Session, except as specific authority for such action has been previously granted by the House of Delegates.

Section 3. Any person who is, and for at least three years prior to election or appointment has been, an active member of this Society shall be eligible to serve on the Board of Directors.

Section 4. Method of Election.

(a) Two members of this Society shall be elected annually to the Board of Directors by the House of Delegates each to serve for a term of three years.

(b) The Nominations Committee shall prepare two slates of nominees, one for each vacancy on the Board. Nominations may be made from the floor. The qualifications of all persons nominated from the floor shall be presented to the House of Delegates at the time such nominations are made.

(c) Election shall be conducted by the Elections Committee.

(d) Election shall be by ballot, voting continuing until a candidate receives a majority vote.

Section 5. The term of office of any member of the Board of Directors elected at an Annual Session shall begin with the sine die adjournment of the House of Delegates at such Annual Session.

Section 6. The president of this Society shall serve as chairman of the Board of Directors and the recording secretary shall serve as secretary of the Board.

Section 7. The president or president-elect and seven other voting members of the Board shall constitute a quorum.

Section 8. The annual meeting of the Board of Directors shall be held within two weeks prior to the first meeting of the House of Delegates at each Annual Session, the time and place of such meeting being designated by the chairman.

Section 9. In the event of removal by death, resignation or total disability of a member of the Board of Directors who is not an officer of this Society, the president of this Society shall appoint one of the candidates from the slate of nominees for members of the Board of Directors from the preceding election to serve until the next Annual Session. At the next Annual Session the vacancy shall be filled by election by the House of Delegates.

Section 10. The method of carrying on the business of the Society between the regular annual meetings of the Board of Directors shall be:

(a) The material shall be submitted in writing in two copies, one to the

president, the second to the executive office for filing. Full reasons for the proposed action shall be stated, and the proposal shall be accompanied by the second of another member of the Board.

(b) Within fifteen days of receipt of material, the president shall submit the proposal, accompanied by the reasons, to the executive office with instructions that it be sent to the Board of Directors for their approval and returned to the executive office.

(c) The results of the vote of the Board of Directors shall then be sent to each Board Member by the executive office.

ARTICLE XI
House of Delegates

Section 1. The membership of the House of Delegates shall consist of the members of the Board of Directors, the members of the Advisory Council and the delegates elected by their constituent societies or appointed in accordance with the provisions of these By-Laws.

Section 2. Each constituent society shall hold an annual election for the purpose of the election of delegates to represent the members of said constituent society at annual or special sessions of the Society.

Section 3. (a) The basis of representation is as follows: One delegate and one alternate for each 25 active members, or major fraction thereof of the constituent society. When the membership of a constituent society exceeds 250 active members, the basis of representation shall be one delegate and one alternate for each 50 active members, or major fraction thereof, above 250.

(b) The number of delegates to which each constituent society is entitled shall be determined by the number of active members who have paid current dues to and are in good standing with this Society. A member who has transferred from one constituent society to another shall be counted as a member of the geographical area in which he holds his membership thirty days prior to the opening of the Annual Session.

(c) Notwithstanding anything to the contrary, each constituent society shall be entitled to at least one delegate and no delegate or delegates representing a constituent society shall have a greater number of votes than the total membership of said constituent society.

Section 4. The secretary of each constituent society shall send to the executive secretary of this Society at least two weeks before the opening of the Annual Session a list of delegates and alternates from his society with their addresses and registry numbers or academic degrees.

Section 5. If the combined number of delegates and alternates present at any Annual Session from any one constituent society is less than the quota of delegates from that society, the president of that constituent society, or in his absence the other officers of that society, shall fill the quota from eligible active members of the constituent society present at the Annual Session. When necessary the president of this Society shall name these delegates.

Section 6. Each delegate and each alternate shall present his credentials to the chairman of the convention Credentials Committee when he registers at the Annual Session.

Section 7. The members of the Board of Directors and of the Advisory Council and the officers of this Society shall not be included in the list of elected or appointed delegates from a constituent society to the House of Delegates.

Section 8. Delegates and alternates shall serve from the time of the first meeting of the House of Delegates at the Annual Session for which they were elected as delegates and alternates to the first meeting of the House of Delegates at the next ensuing Annual Session.

Section 9. A quorum of the House of Delegates shall be a majority of its accredited members registered at the Annual Session.

Section 10. Any member in good standing in this Society is eligible to

attend the annual meetings of the House of Delegates and to participate in discussion but the privilege of the floor shall be granted to a non-delegate only by consent of the House. Only members of the House of Delegates may vote.

ARTICLE XII

Advisory Council

Section 1. (a) The Advisory Council of this Society shall consist of those persons who are the presidents and presidents-elect of the contituent societies or, in the absence of a president-elect such other person as may be selected by the constituent society and those persons who are the members of the Board of Directors of this Society as of the day immediately following the sine die adjournment of the House of Delegates at any Annual Session. These same persons shall remain on the Advisory Council until the sine die adjournment of the House of Delegates at the next ensuing Annual Session even though in that period the presidents of constituent societies on the Council have become the past presidents and the presidents-elect have become presidents of their respective constituent societies.

(b) With the exception of the members of the Board of Directors, it shall be the privilege of a member of the Council to appoint any other active member of his constituent society as his alternate when he is unable to be present at a meeting of the Council, provided that only two active members from a constituent society shall be allowed certification as members of the Advisory Council at any one Annual Session of this Society.

Section 2. The purpose of the Advisory Council shall be to consider matters of importance pertaining to the interest and welfare of the members of this Society and of the members of the profession of medical technology in general. The Advisory Council shall have no power to act for the Society. It may make recommendations to the House of Delegates. By means of a report made by the chairman or other duly authorized member of the Council to the House of Delegates it may bring matters of importance to the attention of the House of Delegates.

Section 3. Officers of the Advisory Council shall consist of a chairman, a vice-chairman, a recording secretary, an assistant recording secretary and a corresponding secretary.

(a) Officers of the Advisory Council shall be elected at a meeting following soon after the sine die adjournment of the House of Delegates at each Annual Session.

(b) No member of the Board of Directors shall hold office in the Advisory Council.

(c) The retiring chairman of the Advisory Council shall call a meeting of the Advisory Council soon after the sine die adjournment of the House of Delegates for the purpose of organizing the Advisory Council. He shall act as chairman pro tem of this meeting. He shall read, or distribute in writing, to the members of the Council a list of the persons eligible for office. Nominations shall be made from the floor. Election shall follow. A majority vote of all members of the Advisory Council present at that meeting of the Council shall elect.

(d) A quorum of the Advisory Council shall be a majority of its members.

(e) Officers shall take office immediately after their election and they shall remain in office until the sine die adjournment of the House of Delegates at the next ensuing Annual Session.

Section 4. The duties of the officers of the Advisory Council shall be as follows:

(a) The chairman shall preside at all meetings of the Council. He shall provide for the preparation and presentation of reports and recommendations from the Advisory Council to the House of Delegates.

(b) The vice-chairman shall in the absence of the chairman assume the duties of the chairman.

(c) The recording secretary shall take the minutes of the meetings of the Advisory Council and shall keep a permanent record of these minutes. He shall, within thirty days after the close of the Annual Session, prepare and distribute to the members of the Advisory Council a copy of the minutes of the meetings held by the Council at the time of the Annual Session just closed.

(d) The assistant recording secretary shall in the absence of the recording secretary assume the duties of the recording secretary.

(e) The corresponding secretary shall carry on the correspondence for the Advisory Council.

Section 5. Any member in good standing in this Society shall be eligible to attend the meetings of the Advisory Council and to participate in discussion, but the privilege of the floor shall be granted to a non-member of the Council only by consent of the Council. Only members of the Council may vote.

(a) Each constituent society shall be entitled to two and only two voting members at any meeting of the Council.

Section 6. The routine operating expenses of the Advisory Council shall be included in the annual budget of this Society. Any request for an appropriation for special activities of the Council shall be presented to the Board of Directors for approval.

ARTICLE XIII

Representation on the Board of Registry

Section 1. The president, the president-elect and the immediate past president of this Society shall represent the Society as members of the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists.

ARTICLE XIV

Representation on the Board of Approved Schools

Section 1. Three representatives of this Society shall be elected by the House of Delegates to serve on the Board of Approved Schools of the American Society of Clinical Pathologists. Nominations shall be made by the Nominations Committee and election shall be by ballot. A majority of the votes cast elects.

Section 2. Persons who are, and for at least five years prior to election or appointment have been, active members in good standing of this Society shall be eligible for election to the Board of Approved Schools provided, however, that such persons shall hold academic degrees at least at a baccalaureate level and have had at least three years of teaching experience within the current decade in a clinical laboratory or in a school of medical technology approved by the Council on Medical Education and Hospitals of the American Medical Association and that they shall have held office either in this Society or in one of its constituent societies or have held membership on the Board of Directors of this Society or on a comparable Board of a constituent society.

Section 3. A representative to the Board of Approved Schools shall be elected to this position for a term of three years. One representative shall be elected at each Annual Session. In 1953 three representatives shall be elected, one to serve for one year, one for two years and one for three years.

Section 4. A representative to this Board shall not serve for more than two terms or more than six years.

Section 5. In the event of the death, resignation or total disability of a representative of this Society to the Board of Approved Schools the Board of Directors shall fill the unexpired term by appointment.

ARTICLE XV

Elections Committee

All elections by the House of Delegates shall be conducted by an Elections Committee consisting of five delegates appointed by the chair. Each

member of the Elections Committee shall receive and carry out the list of instructions previously prepared by the chair in accordance with the parliamentary authority of the Society.

ARTICLE XVI
Special Committees

Section 1. There shall be a Nominations Committee composed of seven active members of this Society, no two of whom shall be from the same constituent society and none of whom shall be an officer of this Society.
(a) The House of Delegates shall elect the Nominations Committee. Nominations for membership on this committee shall be made only from the floor of the House. The time for these nominations shall be fixed for the first day of the meetings of the House of Delegates at each Annual Session, and each constituent society shall be entitled to make only one nomination. The time for election of the Nominations Committee shall be fixed for the second day of the meetings of the House of Delegates at each Annual Session.
(b) Election shall be by ballot, and each delegate shall be provided with a printed ballot bearing names of all nominees. The seven persons receiving the highest number of votes shall be considered elected. The person receiving the highest number of votes shall serve as chairman of the committee.
(c) In the event of a vacancy due to death, resignation or refusal to serve, the Board of Directors shall elect by majority vote an eligible member of this Society to fill the vacancy. The remaining nominees for this committee shall be considered first, in the order of the highest number of votes received.
(d) The Nominations Committee shall provide for the publication in the September-October issue of the official publication of this Society the names and offices of those officers, directors, and representatives for the Board of Approved Schools whose term of office expires at the next Annual Session.
(e) It shall solicit from the constituent societies or from any active member of this Society, names and qualifications of qualified candidates for such offices and positions, with instructions that such material be in the hands of the Nominations Committee not later than December 1st. Only the names of members who have consented to serve if elected shall be submitted by a constituent society or by a member of this Society to the Nominations Committee.
(f) The Nominations Committee shall prepare a slate of at least two nominees for each office and other position to be filled by the House of Delegates. Not less than sixty days before the date of the next Annual Session, a list of nominees together with their qualifications shall be distributed to each active member of this Society.

Section 2. Other special committees may be appointed at any time by the president on authority given him by the House of Delegates or the Board of Directors.

Section 3. The president shall appoint the necessary personnel for committees that shall arrange for the Annual Session of this Society.

Section 4. The President shall appoint a Minutes Committee with authority to approve and correct the minutes of the previous Annual Session of the House of Delegates. An abstract of these minutes and of the minutes of the meetings of the Board of Directors held since the first meeting of the House of Delegates of the last Annual Session shall be published and distributed to members not less than 60 days prior to the next Annual Session.

ARTICLE XVII
Standing Committees

Section 1. There shall be the following nine standing committees: Constitution and By-Laws; Education; Legislation; Membership; Public Relations; Research; Service Fund and Finance; Standards and Studies; Recruitment. Each of such committees shall be composed of six members, with such

geographical distribution as shall be conducive to effective work.

Section 2. At the beginning of his term of office the president of this Society shall appoint, each for a term of three years, two members to each of the standing committees to succeed the two members whose term ended with the sine die adjournment of the House of Delegates at the Annual Session. The president shall appoint a chairman for each standing committee for a term of one year. A member may not serve for more than six consecutive years on the same standing committee. Only active members may be appointed to membership on standing committees.

Section 3. The duties of the standing committees shall be as follows:

(a) The Constitution and By-Laws Committee shall:

(1) Examine all proposed amendments to the By-Laws of this Society. Proposed amendments to the By-Laws of this Society may be submitted by an active member of this Society or by a constituent society. After the Committee makes such changes as are necessary to put these proposed amendments in proper form without altering their intent, it shall have them distributed to each active member of this Society not less than sixty days prior to the next ensuing Annual Session of this Society.

(2) Thirty days after the adoption of an amendment or amendments to the Articles of Incorporation or By-Laws of this Society, the recording secretary shall send an official report of such adoption to the executive secretary who shall provide for publication of the announcement of adoption of such amendments in the September-October issue of the official publication of this Society.

(3) The Constitution and By-Laws Committee shall examine the proposed Constitution, or Articles of Incorporation, and By-Laws of any group petitioning for a charter from this Society and shall bring a report and a recommendation concerning the chartering of such group to the House of Delegates at the next ensuing Annual Session of this Society.

(4) The Constitution and By-Laws Committee shall examine all proposed amendments to the Constitution and By-Laws of a constituent society. It shall submit the same to the chairman of the Board of Directors with an opinion only if a majority of the committee members disapprove the proposed amendments. In such case these must be approved by a majority of the Board of Directors before the proposed amendments are presented to the membership of the constituent society to be voted on in the manner prescribed by the By-Laws of said society.

(b) The Education Committee shall develop teaching aids, encourage the establishment of laboratory libraries, prepare and make available to members and to constituent societies current lists of visual aids, study the curricula of approved schools of medical technology, make suggestions and receive such from the Board of Approved Schools, and aid graduate and undergraduate students of approved schools of medical technology in every way possible.

(c) The Legislation Committee shall assume the duties assigned to it by the House of Delegates and Board of Directors.

(d) The Membership Committee shall plan ways and means of cooperating with the membership committees of constituent societies with the aim of increasing the membership of this Society and of the constituent societies.

(e) The Public Relations Committee shall, by dispensing information and promoting public understanding of the profession of medical technology, act as a liaison group between this Society and other professional or lay groups.

(f) The Research Committee shall direct the research activities of this Society.

(h) The Service Fund and Finance Committee shall stimulate fund raising for service to the undergraduate and graduate medical technologist and shall comply in so far as possible with the suggestions of the Board of Directors

and House of Delegates regarding policies and practices relative to the use of the fund. Disbursements from the fund shall be made by voucher signed by the chairman of the Board of Directors. A separate financial account shall be kept for the fund. This shall be audited as provided for the other financial accounts of the Society. At each Annual Session of this Society an itemized statement of all receipts and expenditures to the fund during the past year shall be presented to the House of Delegates. The Service Fund and Finance Committee shall also study ways and means of meeting allotments established in the budget and shall prepare a budget for the coming year to be presented to the House of Delegates at each Annual Session.

(i) The Standards and Studies Committee shall study medical technology ethics, economics, standards and other kindred problems. This Committee shall inform the members of this Society of the activities of other professional medical technology organizations. It shall promote better understanding of professional medical technologists by the public so that the public may discriminate between adequately and inadequately trained members of our profession.

(j) The Recruitment Committee shall have the responsibility of recruiting students into the field of medical technology.

ARTICLE XVIII
Fiscal Year

The fiscal year shall be from July 1 to June 30, inclusive.

ARTICLE XIX
Official Publication

Section 1. The official publication of this Society shall be THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY, the name of which is patented and the contents of which shall be copyrighted.

Section 2. The official publication shall be made available to all active, affiliate, and student members of this Society who have paid their dues and are in good standing, and to all honorary members.

Section 3. The Editor of the official publication shall be elected by the Board of Directors and shall serve under written contract.

Section 4. The Editorial Board shall consist of the Editor, two or more Advisory Editors and two or more Associate Editors. The Advisory and Associate Editors shall be appointed annually by the Editor with the approval of the Board of Directors.

Section 5. All editorials shall be approved by the Editor-in-chief and one other member of the Editorial Board before publication.

Section 6. All papers read at convention or submitted to the Society shall become the property of the Society and shall be published, if warranted, in THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY under the copyright of the society unless a petition for the privilege of prior publication has been obtained of the Editorial Board.

ARTICLE XX

The executive office of the Society shall be located at Houston, Texas.

ARTICLE XXI
Parliamentary Authority

Robert's Rules of Order Revised shall govern the business proceedings of this Society except when otherwise specified in these By-Laws.

ARTICLE XXII
Amendments

Section 1. The By-Laws of this Society may be amended as follows:

(a) Any proposed amendment to these By-Laws recommended by either a constituent society or by an active member of this Society shall be submitted in writing to the chairman of the Constitution and By-Laws Committee not

later than December 1st. Twelve copies of the proposed amendment in context shall be submitted. The Constitution and By-Laws Committee shall make such changes as may be necessary to provide proper form without altering the meaning of the amendment. After such changes have been made the proposed amendment shall be distributed to each active member of this Society not less than sixty (60) days prior to the opening of the next Annual Session, at which Session the proposed amendment shall be presented to the House of Delegates for vote.

(b) Amendments to these By-Laws shall be adopted by a two-thirds vote of the House of Delegates, except that a proposed amendment to Article XX of these By-Laws shall be approved by a two-thirds vote of the Board of Directors before it may be submitted to the House of Delegates for vote.

Section 2. A complete revision of these By-Laws shall be presented to a meeting of the House of Delegates at an Annual Session only upon two-thirds vote of the House of Delegates at one Annual Session ordering a revision of the By-Laws for presentation to a meeting of the House of Delegates at the next ensuing Annual Session. A two-thirds vote of the House of Delegates shall be necessary for adoption of a revision of these By-Laws.

Section 3. Manner of voting on amendments to Articles of Incorporation:

(a) Amendments shall be made only at an Annual Session of the House of Delegates by a majority vote of the total number of its accredited members. The vote shall be by roll call.

(b) In the event that the authorized quota of accredited delegates of any constituent society is unable to be present at the Annual Session of the House of Delegates, such constituent society shall be entitled to send the duly certified votes of its absent accredited delegates to the Executive Secretary 10 days before the date of said Annual Session, such votes to be cast at the time the roll call vote is taken.